Method Development for the Quantification of Free, Interstitial, Surface, and Bound Water Content in Granular Sludge as a Predictor for Dewaterability

By

Theresa Dessalegn Kopper

Submitted to the graduate degree program in Civil & Environmental and Architectural Engineering and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Science.

_____

Chairperson: Belinda S.M. Sturm

_____

Edward Peltier

_____

Susan Williams

Date Defended: Dec. 13th, 2017

The Thesis Committee for **Theresa Dessalegn Kopper**

certifies that this is the approved version of the following thesis:

Method Development for the Quantification of Free, Interstitial, Surface, and Bound Water Content in Granular Sludge as a Predictor for Dewaterability

_____

Chairperson: Belinda S.M. Sturm

Date approved: Dec 13th, 2017

Method Development for the Quantification of Free, Interstitial, Surface, and Bound Water Content in Granular Sludge as a Predictor for Dewaterability

Abstract

By

Theresa Dessalegn Kopper

Abstract

The purpose of this study was to develop a method to understand the relationship between granular sludge and its distribution of water with respect to dewatering potential. Thermogravimetric analysis (TGA) was optimized to quantify free, interstitial, surface, and bound water. TGA tests were performed for both granular sludge sampled from pilot reactors (70L) and activated sludge fed with the same primary effluent wastewater. The impact of temperature, sample preparation, and sample type on the measured results were evaluated. Activated Sludge contained more free and interstitial water when compared to granular sludge regardless of preparation method; however, granular sludge contained higher amounts of surface and bound water. Additionally, mechanical dewatering of granular sludge had a higher impact on the decrease of interstitial water, when compared with activated sludge. This method was also used to compared granular and flocculent sludge sampled from the same pilot reactor, and the results mirrored those of granular sludge and activated sludge, by revealing higher percentages of free and interstitial water for flocculent sludge, and higher surface and bound water in granular sludge. Filterability was measured in terms of capillary suction time (CST) tests and then

correlated with water distribution data obtained from TGA tests. Free and interstitial water within granular sludge showed a deterioration in the filterability. Moreover, granular sludge had improved filterability when compared with activated sludge; mostly due to the enhanced liquid solid separation established by tightly bound EPS (TB-EPS).

Polymer dose testing was additionally performed to understand the difference between polymer demands for aerobic granular sludge and activated sludge. Granular sludge exhibited substantially less of a demand for polymer, while also producing a higher % cake after mechanical dewatering. Samples before and after conditioning were tested using the TGA method established in this study. It was determined that although water was removed after conditioning, which produced a higher % cake, activated sludge exhibited an increase in surface and bound water after polymer addition. This may result from increased surface area associated with sludge flocculation and excess chemical binding induced by the cationic polymer. Granular sludge required substantially less polymer, and did not yield an observable increase with surface water. However, an increase in bound water was also established for granular sludge after polymer addition.

Phase two of this study provided a fundamental understanding regarding some of the physico-chemical properties that affect the dewatering process of aerobic granular sludge. The findings indicated that higher tightly bound extracellular polymeric substances (TB-EPS) enhanced the overall sludge-water separation process. However, loosely bound EPS (LB-EPS) had a negative impact on filterability due to an increase of free and interstitial water after TGA evaluation. Particle sludge size provided another factor influencing this process, with increases in surface water associated with increased particle diameter. Nevertheless, an improvement in filterability was correlated with increasing particle diameter.

Overall, the purposed TGA method used to quantify the composition of different water fractions within granular sludge gives researchers and utilities the ability to take a closer look at the complex relationship between water and sludge; which can provide additional insight regarding the selection of appropriate sludge handling methods for dewatering granular sludge.

Contents

List of Figures

List of Tables

SYMBOLS

SBR	- sequencing batch reactor
AGR	- aerobic granular reactor
ALE	- alginate like exocellular polysaccharides
cm	- centimeter
COD	- chemical oxygen demand
CST	-capillary suction time
rbCOD	- readily biodegradable chemical oxygen demand
sbCOD	- slowly biodegradable chemical oxygen demand
sCOD	- soluble chemical oxygen demand
pCOD	-particulate chemical oxygen demand
ESS	- effluent suspended solids (mg L^{-1})
EPS	- extracellular polymeric substances
L	- liter
m	- meter
min	- minute
mL	- milliliter
MLSS	- mixed liquor suspended solids (g L^{-1})
SRF	-specific resistance to filtration
TGA	- thermogravimetric analysis
VSS	- volatile suspended solids
PS/PN	- polysaccharide to protein ratio

rpm	- rotations per minute
s	- second
SRT	- sludge residence time (days)
SBR	- sequencing batch reactor
SVI	- sludge volume index (mL g^{-1})
BNR	-biological nutrient removal
PAOs	phosphate accumulating organisms

Acknowledgements

Dr. Belinda S.M. Sturm, my advisor, for introducing me to the realm of wastewater research which has thoroughly captured my interest.

Dr. Edward Peltier and Dr. Susan Williams, for being part of my committee and their guidance in my research and study.

Rasha Faraj, whom I have worked alongside for more than four years. A friend and teacher, thank you for all the time you have invested in me.

To the Bill and Melinda Gates Foundation, for funding me throughout my undergraduate and graduate education. Thus, allowing me to pursue academia wholeheartedly without worry of inadequate finances.

The McNair Scholars program, for encouraging me to start research and equipping me with the tools necessary to succeed in the world of academia.

To the staff at the Kansas River Wastewater Treatment Plant for building the pilot reactors at the WWTP and allowing us students to work alongside their employees in the newly constructed lab, and never failing to lend a helping hand.

To my peers and cohorts in the Sturm lab group, for the wonderful support group we have established amongst each other, and the many laughs we have shared.

My husband, Michael Kopper, for always encouraging me to satisfy my curiosity for wastewater sludge, and for helping me countless times with carting and collecting wastewater.

My sisters, Karina Gabriel and Rosaleen Cabano, for never letting me fall astray in my dreams, while continuing to keep me grounded and humble. As well as, all my nephews, who never cease to remind me of how wonderful this world is.

My father and mother, whom left their lives and families in Ethiopia and Belarus to give me and my sisters better chances in life. I will always marvel at how brave and frightening that was.

1.0 Introduction

Granular sludge is beginning to make its debut into the US market as a replacement for the traditional activated sludge process. Granular sludge exhibits simultaneous nitrification and denitrification, as well as phosphorus removal, within one reactor due to the unique biofilm structure. Whereas, conventional activated sludge typically needs a series of reactors operating with costly return flows to sustain environments suitable for nutrient removal (De Kreuk et al. 2005, Winkler et al. 2011). The improved settling characteristics of granular sludge also make it advantageous to the slower settling activated sludge, which history has proven to be susceptible to washouts during heavy rain events. Although granular sludge has been intensely investigated in the liquids train, the solids handling aspect is lacking for utilities whom wish to adopt this process. Differences in physico-chemical properties of granular sludge pose the question of whether granular sludge will have considerable deviations when leaving the liquids train and entering solids handling. A holistic understanding of granular sludge dewatering potential is needed to allow for implementation in full-scale. Traditionally, dewatering of biosolids is considered as one of the most costly and difficult steps in wastewater treatment, and can require a capital and operational cost of approximately 25%-50% of the total treatment process (Karr and Keinath 1978, Murthy and Novak 1998, Neyens et al. 2004). The methods involved for dewatering require energy and chemical conditioners to aid in the removal of water.

Studies have indicated that different water fractions which include: free, interstitial, surface and bound water, are present within activated sludge. These water types require different levels of mechanical, chemical, or thermal energy with regards to removal due to the intensity of their physical or chemical binding (Vesilind 1994, Kopp and Dichtl 2001). Most studies agree

that free water or bulk water can be removed by physical mechanisms, and bound water is the limiting factor when it comes to increasing % cake (Vesilind 1994, Colin and Gazbar 1995, Kopp and Dichtl 2000).

In addition to the water content, extracellular polymeric substances (EPS), which are biosynthetic polymers that aid in the aggregation of microbial cells (Wingender et al. 1999), affect sludge dewaterability. In one study, higher fractions of loosely-bound EPS (LB-EPS) correlated with deterioration in dewatering (Yang and Li 2009). Other studies point to an increase in total EPS resulting in less % dry cake matter due to possible water entrapment within the flocs (Houghton et al. 2001, Mikkelsen and Keiding 2002). Recent research suggests that dewatering of activated sludge improves when flocs contain less singe cells and non-biodegradable EPS. This study also found that polyvalent ions improved floc strength resulting with improved dewatering. However, sodium ions reduced dewaterability by disintegrating the sludge floc (Christensen et al. 2015).

To understand the fundamental nature of water within granular sludge, method development for the quantification of different water forms within granular sludge is required. The purpose of this study is to evaluate the use of thermogravimetric analysis (TGA) for quantifying the free, interstitial, surface and bound water within granular sludge samples taken from pilot reactors fed with primary effluent wastewater. Differences in testing temperature, sample preparation, and sample type were evaluated. Additionally, capillary suction time (CST) tests were performed on samples for an analysis of filterability. Furthermore, interest in the effect of EPS composition and structure on water fractions were explored by comparing dewatering potential for granular sludge with varying EPS concentrations due to changes in F/M ratios. Particle size was also examined to recognize the impact diameter has on water fractions within

granular sludge. Polymer dosage testing for granular sludge was additionally tested and compared with polymer demand for activated sludge.

2.0 Literature Review

2.1 Water Types Found in Wastewater Sludge

To recognize the dewatering potential of granular sludge, knowledge of the different water fractions within wastewater sludge is key. Moisture distribution within activated sludge has been an essential component for the investigation of dewaterability within previous studies (Vaxelaire and Cézac 2004). Water held within the sludge floc does not have the same properties as bulk water due to the presence of a solid particle. The water fractions inside wastewater sludge differ due to changes in density, viscosity, vapor pressure, enthalpy, and entropy (Katsiris and Kouzeli-Katsiri 1987, Vesilind 1994). Therefore, the principal behavior of water is reliant on its closeness to a solid surface.

2.1.1 Free Water

Free water is considered to not be influenced by solids, which include the water in voids that are not affected by capillary forces, allowing it to freely move within the sludge sample (Vaxelaire and Cézac 2004). It is commonly accepted that free water can be removed by mechanical processes. One study indicated that vacuum filtration under 5×10^4 Pa for 30 minutes can remove all free water (Smollen 1990), and another stated that all free water can be removed with high pressures, typically larger than 28 MPa (Pramanik 1994). A large portion of water contained within wastewater sludge is considered free or unbound, and most researchers agree that free water the fundamentally easiest water fraction to remove.

2.1.2 Interstitial Water

Interstitial Water has been classified in literature as water trapped inside crevices and spaces of flocs and organisms, which are bound by capillary forces (Tsang and Vesilind 1990,

Vesilind 1994, Vaxelaire and Cézac 2004). Interstitial water is also held in the floc structure, and can become free water if the structure is destroyed or the interstitial water is mechanically squeezed out (Tsang and Vesilind 1990, Kopp and Dichtl 2000). Studies have shown similar trends with regards to low and high solid contents. It is reported that low solids content result in disintegration of sludge flocs, which lead to the release of interstitial water (Smith and Vesilind 1995). However, high solids content have also been linked to a decrease in interstitial water; with studies suggesting that high solids concentrations lead to floc compression; thus, physically squeezing out the interstitial water (Katsiris and Kouzeli-Katsiri 1987, Robinson and Knocke 1992). Polymer conditioning of sludge also resulted in a decrease of interstitial water, and literature has interpreted this as an exchange of polymer with water on the binding sites of the sludge flocs (Robinson and Knocke 1992). Studies using drying methods to analyze water fractions within wastewater sludge conclude that interstitial water is the moisture after the free water is removed, thus quantified by a decrease in drying rate (Tsang and Vesilind 1990, Vesilind 1994, Kopp and Dichtl 2001, Vaxelaire and Cézac 2004).

2.1.3 Surface Water

Surface water, occasionally referred to as vicinal water, includes water attached to the surface of solid particles by adsorption and adhesion forces (Vaxelaire and Cezac 2004, Vesilind 1994). Since 1957, scientists have studied the effect of solid surfaces on water molecules and determined water molecules tend to layer themselves on solid structures, and contain properties which differ from the bulk liquid due to structural modification induced by closeness to a surface (Drost-Hansen 1981). These layers are believed to have strong bonds associated with Van der Waals forces due to the presence of a solid surface, and the structure requires more energy to break those bonds. The extant of the surface bonding decreases as the distance from the surface

increases (Vesilind 1994). Removal of layered water molecules in close proximity to solid surfaces is considered to have solvation forces which extend to approximately 3 nm from each solid surface. Increasing energy is needed to remove each layer of water molecules as the surfaces converge, and at these distances van der Walls forces are minor when compared to hydrogen bonding (Chan and Horn 1985, Israelachvili and Kott 1989, Vesilind 1994).

Surface water can also be held within the surface of cells. The main difference between surface and interstitial water is that interstitial water has the freedom to move when physical confinement is removed (Vesilind 1994). Hence, unless the surface of the sludge particle or microbial cell is destroyed, surface water will require large amounts of thermal energy to effectively remove.

2.1.4 Bound Water

The literature differs in the classification of bound water due to different measurement techniques. However, most studies agree that bound water is chemically bound to particles. Some studies refer to chemically bound water as water of hydration (Halde et al., 1979, Vesilind 1994, Tsang and Vesilind 1995, Smith and Vesilind 1995), while others discuss bound water as intracellular water (Kopp and Dichtl, 2001) or internal water (Lee and Lee 1995). However, all agree that bound water is chemically bound to solid particles, and can only be removed using thermal energy (Vesilind 1994, Mahmoud 2010). Similar to surface water, bound water cannot be removed mechanically. Therefore, both are considered the limiting factors in dewaterability of wastewater sludge (Kopp and Dichtl, 2001, Vesilind 1994).

Methods to quantify bound water in wastewater sludge vary from drying tests (Smollen 1990, Halde 1979, Kopp and Dichtl, 2001), dilatometric tests, which use instruments that

measure the change in volume of a solution when a physical or chemical stress is exerted upon the sample (Wu et al., 1998, Smith and Vesilind, 1994, Robinson and Knocke, 1992, Heukelekian and Weisber, 1956), centrifugal tests (Colin and Gazbar 1995, Lee and Hsu 1995), filtration tests (Li and Yang 2007), differential thermal analysis (DTA) (Katsiris and Kouzeli-Katsiri 1987), differential scanning calorimetry (DCS) (Lee and Hsu, 1995), and a combination of thermogravimetric analysis with differential thermal analysis (TGA/DTA) (Chen et al. 1996, Wu et al. 1998, Vaxelaire and Cézac 2004). These tests employ the use of physical (i.e. centrifugation and filtration) or thermal processes (i.e. drying tests), to quantify the bound water content. It is important to note that there are concerns in the scientific community with the clarification of bound water, and what technique truly characterizes the water of hydration.

2.2 Typical Dewatering Processes

Dewatering processes have been tried and tested for primary activated sludge and waste activated sludge, however, selecting an appropriate dewatering process to remove water from granular sludge will require bench scale and pilot scale testing due to the different removal efficiencies associated with each process, and the physio-chemical differences of granular sludge when compared to activated sludge. Previous studies have shown that variations between different wastewater treatment plant sludge's result in different dewatering potentials (Christensen et al. 2015, Skinner et al. 2015), therefore, granular sludge's unique composition is bound to provide the scientific community with extra questions regarding dewatering potential. Process selection for dewatering of granular sludge will likely be the next avenue of research associated with granular sludge and its dewaterability. However, process evaluation will not be included in this scope of study.

2.2.1 Typical Operations

A vast array of dewatering processes are available for full-scale use. However, understanding the different types of water associated with wastewater sludge is needed to understand the effectiveness of each method. Free and some interstitial water may be removed by physical means, while electro-dewatering can be utilized to remove some interstitial and surface water. Thermal drying is required to remove a majority of the surface water and bound water (Mahmoud et al. 2010, Techobanoglous et al. 2014). Some research also indicates that the destruction of surface area on a sludge particle is ultimately needed to remove surface and bound water (Vesilind 1994). With these considerations, wastewater treatment plants should select a dewatering process that removes the highest amount of water, while minimizing the cost. Some methods for dewatering include the belt filter press (BFPs), centrifuges, rotary presses, screw presses, recessed-plate filter presses, electro-dewatering, drying beds, lagoons, vacuum filter and thermal drying.

2.2.2 Belt-Filter Presses

Belt filter presses (BFPs) require little energy and have low capital and operational costs. The maintenance and operations of a belt press is relatively simple. Utilizing high pressures, the process can produce very dry cake. However, belt presses are hydraulically limited with regards to quantity treated, and they may require a sludge grinder before it hits the belt. BFPs are also sensitive to sludge feed characteristics and have a short media life when compared to other mechanical devices using cloth. The use of a gravity thickener is sometimes necessary to remove a large portion of the free water. Resultant cake solids range based on polymer addition and pretreatment; however, ranges of 11-30% cake solids are typical (Techobanoglous et al. 2014).

2.2.3 Centrifuges

Centrifuges are efficient when it comes to removing a large amount of water and consequently producing a very dry cake. The startup and shut down is quick with respect to other mechanical processes and the capital cost is low. However, operational costs exceed traditional presses, and the maintenance requires skilled personnel. Centrifuges may also entail grit removal and sludge feed grinders to maintain operations, and the centrate is typically high in suspended solids. Polymers are dosed in the range of 1.0-25 g/kg of activated sludge, and can reach as high as 30% solids content with the use of polymer and conditioning agents (Techobanoglous et al. 2014).

2.2.4 Rotary Presses

Rotary presses work with low speeds which decreases operational costs; however, the enclosed designs traps odors and aerosols. When using polymer, overdosing does not pose an issue with regards to clogged screens, therefore the dewatering is not hindered. Nevertheless, a large footprint is needed and there are typically volume limitations, which result in multiple units. Screw presses operate very similarly to rotary presses; yet, one additional consequence is that wash water is periodically needed during the operating cycle. Final cake solids are dependent on polymer usage and pretreatment is similar to dry rotary presses (Techobanoglous et al. 2014).

2.2.5 Recessed Plate Filter Presses

Recessed plate filter presses produce the highest dry cake concentration with low suspended solids in the filtrate. The operation is simple, and the rate of solids capture is high.

Nevertheless, this batch process requires expensive equipment and skilled labor costs, and a support structure due to its heavy weight (Techobanoglous et al. 2014).

2.2.6 Electro-Dewatering

Electric field-assisted dewatering, also known as Electro-Dewatering is a new process which exhibits promising results for difficult biosolids. This process is roughly three to five times more energy efficient than traditional dryers, and has the advantage of pathogen destruction during operations. Operations of an electro-dewatering unit is fully automated and has flexibility with regards to differing influent sludge characteristics. Electro-Dewatering involves the phenomena of electrophoresis, electro-osmosis, and electro-migration (Mahmoud et al. 2010). The disadvantages of this newer technology include high capital costs and a need for approximately 10-25% solids before entering the feed stream. Bench-scale testing is required to predict performance. Outlet cake solids can be as high as 49% solids, with an average of 30% for sludge and biosolids (Mahmoud et al. 2010, Techobanoglous et al. 2014).

2.2.7 Drying Beds

Unlike Electro-Dewatering, drying beds have been applied for many years, resulting in a well-known application. Drying beds exhibit the lowest capital and operation cost; however, a large footprint is needed. Influent sludge variability has less of an impact with this dewatering method, and there is no need for chemical addition to aid in the process. The drawbacks of this method include: large footprint, necessity for stabilization to reduce the number of pathogens, and moderate arid climates preferred. Locations receiving a large amount of sun exposure are best suited, but enclosed drying beds can serve year-round in variable weather (Techobanoglous et al. 2014).

2.2.8 Lagoons

Similar to drying beds, lagoons have drawbacks regarding land requirements, climate considerations, and vector attraction. However, this process is also low maintenance, low energy and the capital cost is small. Additional problems resulting from the use of sludge lagoons are the potential for groundwater contamination from leachate if not properly lined (Techobanoglous et al. 2014).

2.2.9 Thermal Drying

As mentioned, removal of bound water must involve the use of thermal drying. Thermal drying utilizes heat to evaporate moisture and reduce the total water content greater than traditional dewatering methods. Some advantages of this process are that it is has a small footprint and produces class A bio-solids with no chemical addition. The reduction of water within the sludge also raises the heating value of the biosolids, which is an attractive option when considering energy capture from biosolids. The disadvantages associated with thermal drying are that the capital costs are high, along with larger operational costs due to fuel consumption (Wang et al. 2010). Highly trained staff are required to operate the system, and there is an increased explosion and fire risk. Dust and odor problems may also arise (Bennamoun et al. 2013, Techobanoglous et al. 2014)

2.3 Stabilization and chemical Conditioning of Wastewater Sludge

Removing water from wastewater sludge is a necessity due to the costs associated with transport and tipping. The removal of excess water also increases the caloric value prior to incineration. If the biosolids are land applied, reducing the amount of water also decreases the

amount of potential leachate. There are many additional benefits to dewatering; however, a balance between costs and % dry cake is ultimately the deciding factor (Vaxelaire and Cézac 2004, Techobanoglous et al. 2014). Sedimentation of primary and waste activated sludge is a typical precursor to mechanical dewatering, and this generally achieves a % solids of approximately 1.5-4 % solids. Mechanical dewatering processes can reach up to 20-30% solids, but this is with the addition of polymers and conditioners (Skinner, Studer et al., 2015). The removal of bound water in wastewater sludge is considered the rate limiting step, since only thermal processes can eliminate this water type. Consequently, complete removal of free water can be considered the most efficient means to produce the targeted solids concentration (Tsang and Vesilind 1990, Kopp and Dicht 2001).

In 2010, the United States, China, and the European Union collectively produced more than 8 million tons of dry solids (Müller 2001). As previously mentioned, wastewater sludge or biosolids (depending on whether stabilized) is typically 1.5-4 % solids before dewatering. Wastewater treatment plants across the world target different solid contents based on land area for application, process operations, and a myriad of other considerations. Resultant solids concentrations based on polymer addition and pretreatment range from 11-30% solids when BFPs are used (Metcalf and Eddy 2014). Centrifuges can also reach up to 30% dry cake solids with polymers dosed in the range of 1.0-50 g-polymer/kg-MLSS. Electro-dewatering, which requires a 10-25% solids content before entering the feed stream, can produce a very high solid content with difficult to dewater sludge; however, bench-scale testing is needed to predict performance. Electro-dewatering has produced cake solids as high as 49%, with an average of 30% solids for untreated and treated sludge and biosolids (Metcalf and Eddy 2014, Mahmoud et

al., 2010). Overall, the degree of dewatering is variable between different wastewater treatment plants and the composition and pretreatment of their biosolids.

2.3.1 Stabilization of Biosolids

Wastewater sludge can undergo sludge stabilization to reduce pathogens and lessen the concentration of offensive odors. Stabilization is performed by the reduction of volatile suspended solids (VSS) content, which reduces the organic fraction of the wastewater sludge. This organic fraction allows microorganisms to survive and develop within certain environments (Techobanoglous et al. 2014). Different sludge stabilization processes provide different qualities of biosolids.

The more commonly used processes are alkaline stabilization, anaerobic digestion, aerobic digestion and composting. Although each process differs in its destruction of pathogens and organic matter, the key factor remains that chemically or biologically, organic matter is destroyed through a series of reactions which change the microenvironments of the wastewater sludge. Wastewater sludge is referred to as biosolids after stabilization occurs. Many wastewater treatment plants choose to stabilize their sludge if land application follows dewatering. Regulations may also cite that biosolids should be Class A or Class B, therefore stabilization is necessary to meet these standards.

2.3.2 Chemical Conditioning

The use of chemical conditioners, generally referred to as polymers, is common in wastewater treatment plants who wish to achieve higher dry solids content after dewatering. However, it is important to note that heat treatment and freeze thaw methods have also been employed to condition sludge or biosolids for improved water removal. Yet, heat and freeze thaw

methods are more common with lab-scale tests and separation of oil and water (Robinson and Knocke 1992, Chen and He 2003, Muller 2001).

Polymers are chains of individual linear monomer units which disassociate to negatively and positively charged species when in contact with water. Wastewater sludge is characteristically negatively charged. Therefore, commercial polymers for wastewater use are cationic (Metcalf and Eddy 2015, WEF 2012). These positively charged long chains aid in the flocculation of suspended and colloidal solids, consequently improving liquid-solids separation. Previous studies indicate that destruction of volatile solids after digestion correlates with an increase in polymer demand by increasing the colloidal fraction of the solution (Novak et al., 2004, Murthy and Novak 1999, Bivins and Novak 2001). Dosage varies between wastewater sludges and type of polymer used. However, studies indicate that ranges from 5-25 g-polymer/kg-sludge is used in most facilities (Abu-Orf and Ormeci 2005). Predicting the dosage of polymers can be accomplished by tests such as the capillary suction time tests (CST), specific resistance to filtration test (SRF), and the standard jar tests (Standard Methods 2012).

The use of polymer is associated with increased operational and capital costs at wastewater treatment plants. Storage and mixing of the sludge or biosolids with polymer is critical when operating. Wastewater treatment plants which desire a high % of dry cake can optimize their polymer dose to save additional funds, while still maintaining a high % dry cake. Costs between different polymers are associated with the relative charge density, molecular weight, electrical charge and molecular structure. (Abu-Orf and Ormeci 2005, Ormeci 2007)..

2.4 Introduction to Granular Sludge

Attention to granular sludge has recently developed due to promising results from bench, pilot and full-scale applications (De Kreuk et al. 2005, Adav et al. 2008, de Kreuk et al. 2010, Faraj 2014, Li et al. 2014, Pronk et al. 2015, Faraj et al. 2017). Granular sludge provides a cost-effective solution for plants that need to expand but have little room for additional footprint. This biofilm process differs from conventional attached systems because aerobic granules form without a carrier. This is accomplished with plug flow regimes under high substrate availability (Adav et al. 2008, Faraj 2014, Sturm et al. 2015).

2.4.1 Nutrient Removal

Nutrient loading to receiving water bodies is an increasing problem, which wastewater treatment plants (WWTP) across the world are attempting to combat. Introduction of oxygen demanding materials to a body of water creates hypoxic zones, consisting of less than 2 ppm of dissolved oxygen. These environments are detrimental to most marine life, and introduction of nutrients such as nitrogen and phosphorus aid in the depletion of oxygen through a series of biochemical reactions resulting from the growth and decay of algae.

The morphology of aerobic granules allows micro-environments of aerobic, anoxic, and anaerobic zones to form. Different bacterial species associated with different redox gradients are selected within the granule, thereby allowing for biological nutrient removal within the granule matrix. This is a stark comparison to biological nutrient removal (BNR) utilizing multiple reactors working in series. This sludge type is an effective application for simultaneous nitrification-denitrification and phosphorous removal, see Figure 1 (Beun et al. 2001, De Kreuk et al. 2005, Wang et al. 2010).

The location of nitrifiers, dentirifiers and polyphosphate-accumulating organisms (PAOs) play a key role in the conversion of nutrients within a granule. Aerobic Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB) are found on the outer edge of the granule where oxygen can penetrate the surface. Denitrifying organisms referred to as denitrifiers, are located predominately in the inner granule and preform conversion of nitrate to nitrogen gas within anoxic conditions. Meanwhile, in the inner granular core, phosphorus accumulation organisms (PAOs) store soluble organic compounds as intracellular polymers (Polyhydroxybutyrate, PHB, or glycogen) (Dulekgurgen et al. 2003). After PAOs oxidize their intracellular storage polymers, they uptake phosphate from the bulk liquid as polyphosphate. Phosphorus is finally removed from the wastewater via sludge wasting. Overall, the granular sludge structure affects the presence of microorganisms by selecting for larger populations which can flourish through the structural support of one another, thus, performing their metabolic capabilities (Winkler et al. 2012).

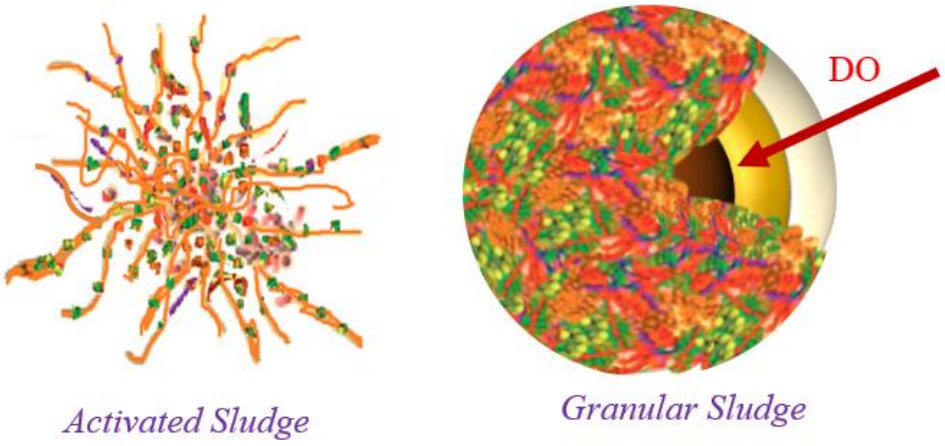

Figure 1: Schematic of physical differences of Activated sludge and Granular sludge. The structure of granular sludge allows for microenvironments suitable for different organisms to perform their metabolic capabilities. The outer core of granular sludge primarily houses AOB's and NOB's while the anoxic inner core selects for denitrifiers and PAO's. The diffusion of dissolved oxygen (DO) is essential in establishing these microbial communities

2.4.2 Settling Characteristics

Morphological differences of granular sludge compared to activated sludge also result in improved settling characteristics. Reported settling velocities for activated sludge range between 7-10 m/hr. Granular sludge has exhibited settling velocities greater than 10 m/hr, making it very advantageous for secondary settling and hydraulic intensification (de Bruin et al. 2004). The sludge volume index (SVI) for granular sludge is typically in the range of 18-75 mL/g biomass, which indicates a higher compactness of settled sludge when compared to activated sludge, with SVIs averaging greater than 75 mL/g biomass (Beun et al. 2001, Tay et al. 2002, De Kreuk et al. 2005, McSwain et al. 2005). Settling characteristics of granular sludge play a role on dewaterability due to the compact settling, which results in increased solid-liquid separation.

2.5 Formation of Granular Sludge and the Role of EPS

2.5.1 EPS Matrix

A granule is a microbial aggregate formed of cells and extracellular polymeric substances (EPS). EPS is composed of polymers which include polysaccharides, proteins, glycoproteins, nucleic acids, phospholipids, humic acids, alginate and alginate-like substances (McSwain et al. 2005, Lin et al. 2010). A crucial factor to initiate and sustain granulation involves applying sufficient substrate for the granular sludge system. This encourages greater production of EPS and microbial storage polymers, which is considered the responsible mechanism for bridging bacterial cells together without a carrier material (Adav et al. 2008). Additional factors which influence the formation and integrity of granular sludge include settling time (Beun et al. 2001, Moy et al. 2002, McSwain et al. 2004), loading rate (Liu et al., 2007; Li et al., 2010; Lee et al., 2010; (Sturm et al. 2015), composition of substrate (McSwain et al. 2005, Yilmaz et al. 2008, Faraj 2014) , shear force (Beun et al. 2001, de Bruin et al. 2004, de Kreuk et al. 2010), and reactor design (Beun et al. 2001, McSwain et al. 2004, Adav and Lee 2008). Furthermore, recent advances in granular sludge show that external and internal selectors are essential in the formation and stability of aerobic granules, particularly with low strength wastewater. An external selector establishes the retention of dense fast settling particles and can be accomplished via wasting with sieves, hydro-cyclones used for density separation, or other physical and mechanical means to retain granular products while wasting fines. Internal selectors based on metabolic properties use the principles behind substrate availability to initiate and sustain granular products. Food to mass ratio is an operational factor that utilities can control for substrate availability. It was found that sufficient substrate availability for granular formation and stability was established by an F/M ratio of up to 0.2 g rbCOD/gVSS-d, which resulted in

approximately 60% granules with a settling velocity higher than 9 m/hr observed in municipal pilot reactors (Faraj et al. 2017).

It is widely accepted that EPS plays a major role when it comes to bioflocculation and dewatering of activated sludge (Liu and Fang 2003). However, understanding dewaterability of granular sludge with respect to the EPS structure and composition is unknown, and this will be investigated in this research.

2.5.2 Structural Characteristics of EPS and the Impacts on Dewaterability

EPS is comprised of biological polymers acting as a glue and possible carbon source for the conglomerated cells. However, studies speculate that the EPS probably has a double-layered EPS structure consisting of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS), which surrounds the cell (Poxon and Darby 1997, Li and Yang 2007). Li and Yang 2007 discovered that LB-EPS negatively affected the bioflocculation and sludge–water separation as a result of the weakened floc structure. Tightly bound EPS (TB-EPS) showed less of a correlation with regards to liquid-solid separation (Li and Yang 2007, Yang and Li 2009). Past studies have also referred to the effect EPS has on dewatering potential; however, many times the focus is on total EPS. One study suggests that high EPS content led to lower shear sensitivity and lower degrees of dispersion, which ultimately led to better filterability (Mikkelsen and Keiding 2002). Granular sludge is known to have more EPS when compared to activated sludge, so it is important to understand the effect of EPS on granular sludge dewaterability.

2.6 Methods to Determine Sludge Dewaterability

2.6.1 Capillary Suction Time Tests

A method widely used to determine the filterability of activated sludge is the Capillary Suction Time (CST) test. Before the use of the CST test, specific resistance to filtration (SRF) was generally applied (Coackley and Jones 1956). The SRF test utilizes Darcy's equation to measure the pressure drop for flow through a porous media. However, the SRF test is disadvantaged by the time intensive nature and is thus not performed regularly by utilities (Novak et al. 1988). Therefore, push towards developing a simple and rapid test for measuring the filterability of wastewater sludge was warranted.

The CST test uses two plastic blocks with a piece of filter paper and two sensor regions using electrical fixed contacts in the upper plastic block. A sample of wastewater sludge or biosolids is poured into the stainless-steel collar which is wedged within both blocks and is flush with the filter paper. When the test is underway, water from the sample begins to flow through the filter paper, forming a cylindrical wet stain moving outward. After flowing 0.8 cm past the stainless-steel collar edge, the water meets the first electrical sensor and begins the timer. As the circular front continues to move, it encounters the second electrical signal which stops the timer. The distance from the first timer to the second is 0.7 cm. The CST measurement is the time it takes for the water front to travel from the first electrical sensor to the second, and it is read in seconds. Wastewater sludge or biosolids having low CST values indicate that water is released quickly from the sample. Sludge with high CST values suggest deteriorated dewaterability (Vesilind 1988). Although the CST is rapid and extremely easy, it does not provide a fundamental understanding of the dewaterability of the sludge sample, and CST values are

unique to specific solids concentrations. Efforts towards correlating CST with SRF have been attempted, but these did not yield beneficial results for modeling CST with SRF (Unno et al. 1983, Vesilind 1988).

2.6.2 Thermogravimetric Analysis

While CST and SRF are used to obtain a sense of the filterability for a sample, methods for quantifying the different fractions of water require intensified procedures and analysis. There are many tests employed to determine the different water fractions held within wastewater sludge, but increased curiosity regarding Thermogravimetric Analysis (TGA) application has been mounting due to the ease of the test and possible prediction of full-scale dewaterability (Kopp and Dichtl 2000, Kopp and Dichtl 2001). Before TGA was applied, drying curves, which are the fundamental principle behind TGA, have been used to quantify the moisture content in wastewater sludge and biosolids for many years (Keey 1978, Smollen 1990, Robinson and Knocke 1992, Pramanik 1994). This method adopts the assumption that the evaporation of water is contingent on the bonding strength between the water molecules and solid surface. Changes with the drying rate of a sludge sample would provide information regarding the moisture distribution. Drying curves for activated sludge typically begin with a short period of increasing temperature and followed by four different rates of drying: (1) a constant rate associated with the free water portion followed by; (2) the first falling rate thought to be interstitial water ; (3) the second decreasing rate related with surface water; and (4) the final portion of the drying curve starting at the end of the second falling rate and ending at an evaporation flux of zero; this final portion is considered to be the drying rate of bound water. In Figure 2, the four main phases commonly associated with drying curves are shown (Keey 1978, Vaxelaire and Cézac 2004).

The first transition between evaporation rates signifies the critical drying point, in which the transfer properties within the sample control the drying rate

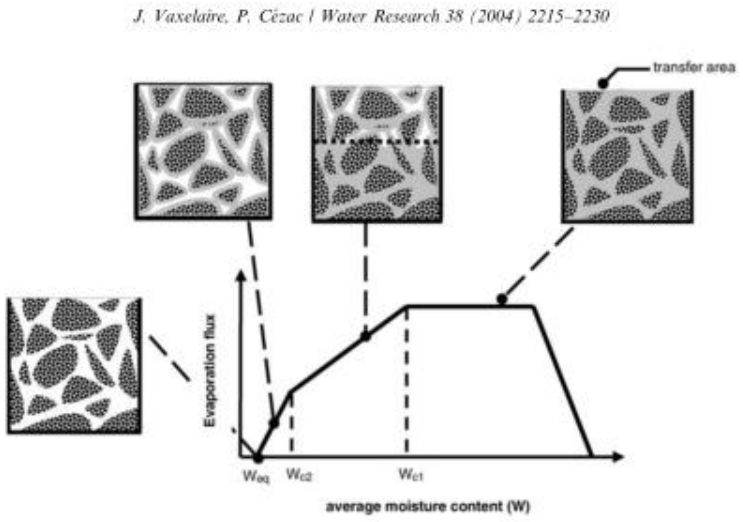

Figure 2: Classic Drying curve taken from (Vaxelaire and Cézac 2004) detailing the critical drying points established by transitions in slopes.

Thermogravimetric analysis of a wastewater sludge samples have been utilized to predict full-scale dewatering results. This was accomplished by performing a drying test using a thermogravimetric analyzer operating at 35º C with a 30 mL/min air flow rate. It was found that the transition at the critical drying rate signified the maximum dewatering potential. This location in the drying curve indicates the moment when increased energy is needed to remove excess water. This study also found that the water binding energy for interstitial water is higher than 5 kJ/kg water, while the binding energy for free water was less than 0.28 kJ/kg water (Kopp and Dichtl 2001). Kopp and Dichtl describe that the first visible change in slopes of drying rate vs. g-water/ g-solid allows for prediction of full scale dewatering due to the increased need in energy to remove additional water. When correlating the solid content remaining at point A of

the drying curve with the solid content after dewatering in full- scale processes using centrifugation and BFPs, the results had a coefficient of determination of 0.925 (Kopp and Dichtl 2000, Kopp and Dichtl 2001), see Figure 3 and Figure 4. These results are very promising for determining the maximum dewatering potential of activated sludge, but granular sludge was not investigated in this study. Therefore, method development for the determination of water fractions within granular sludge is needed to understand the dewatering potential of granular systems.

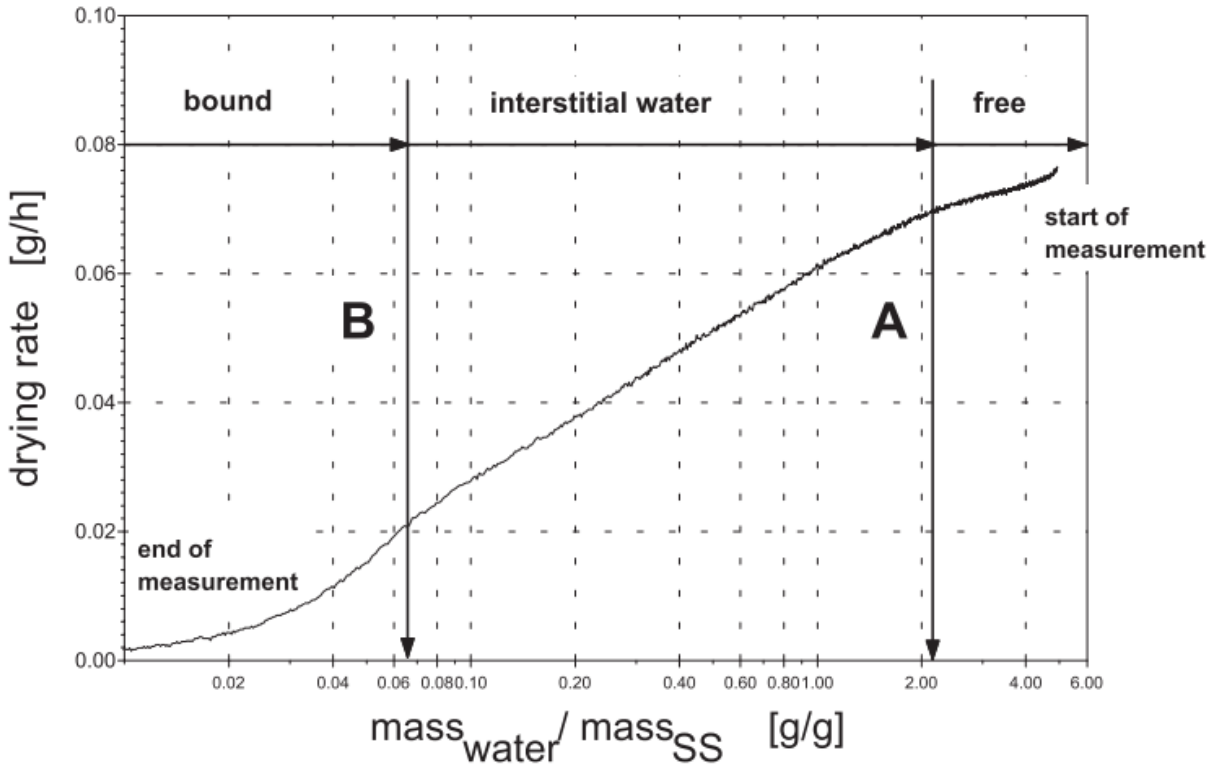

Figure 3: Drying curve established by TGA for activated sludge taken from (Kopp and Dichtl 2001) showing point A of the curve which is described as the critical point were evaluation of full scale dewaterability can be determined based on energy needed for removal of all free water

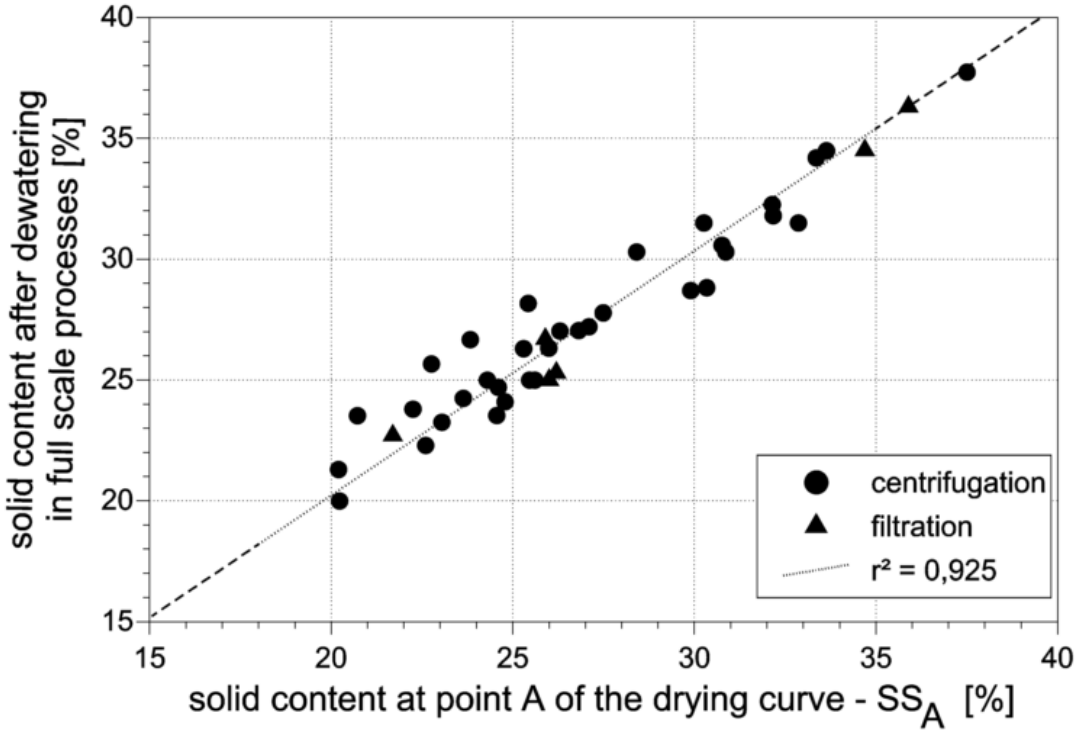

Figure 4: Comparison of solid content at the first transition from free to interstitial water established by the first critical drying point, with solid content after full scale dewatering taken from (Kopp and Dichtl 2001).

2.7 Additional Factors Influencing the Dewaterability of Wastewater Sludge

Granular sludge dewaterability is a topic with much interest, but an understanding of possible factors influencing the dewaterability of wastewater sludge is required to build a holistic understanding of how the granular structure may affect dewaterability. Recently, some wastewater plants utilizing biological phosphorus removal and anaerobic digestion have noticed a deterioration in % cake after dewatering, leading to an increase in polymer demand. Phosphorus accumulating (PAOs) release their stored polyphosphate during anaerobic digestion. This increase in phosphate is believed to influence the cation chemistry of the digestate by complexation and precipitation reactions with calcium and magnesium. This idea supports

divalent cation bridging theory (DCB), which proposes that the ratio of monovalent to divalent (M/D ratio) cations have an impact on dewatering and settling of wastewater sludge, which play a role in cross-linking negatively charged functional groups within the EPS sludge matrix (Higgins and Novak 1997, Higgins et al. 2004).

Researchers found a correlation between the M/D ratio and % cake solids measured in laboratory settings. As shown in Figure 5, as M/D ratios increase, % cake solids decreases (Higgins et al. 2014). Another study found that plants using anaerobic digestion can expect a 3-5% decrease in dry cake solids and a 30% increase in polymer consumption when they transition from chemical P-removal to biological P-removal (Forstner 2016).

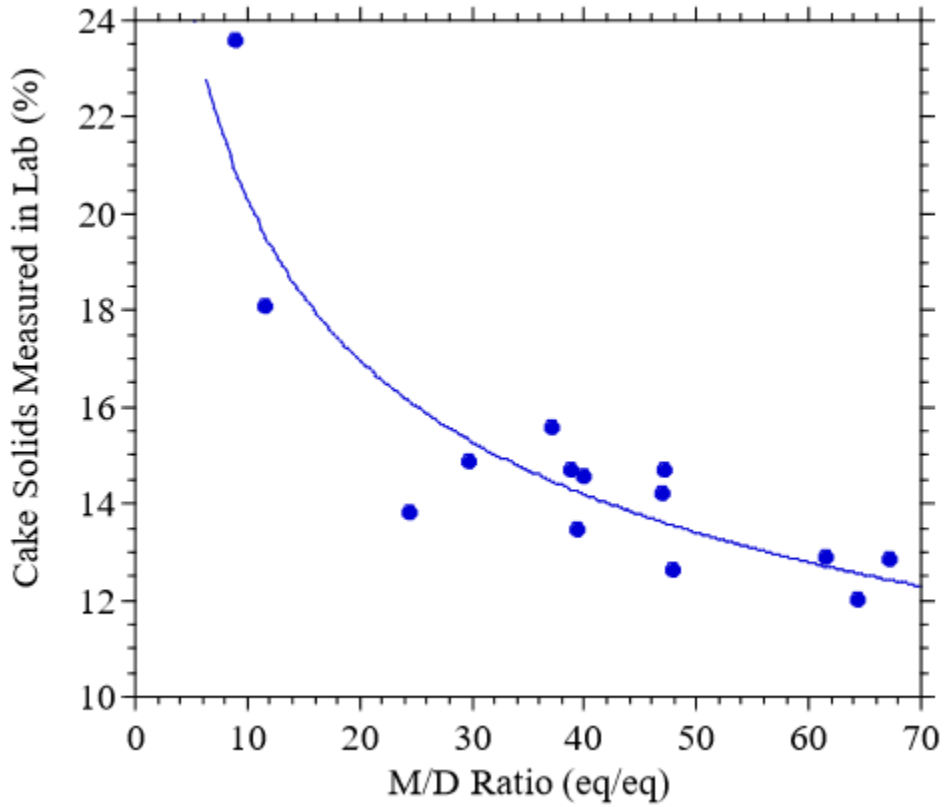

Figure 5: Impact of M/D ration on the Cakes solids measurement for lab tests taken from (Higgins et. al., 2014)

Another factor affecting the dewatering of wastewater sludge is the concentration of bound extracellular protein concentrations. The binding of biopolymers is related to the divalent cation bridge theory. This was tested by adding excess sodium concentrations to a laboratory scale activated sludge reactor. The results showed a decrease in bound protein concentrations, which resulted in a decrease in bioflocculation. The researchers proposed that divalent cations bind extracellular proteins within the sludge floc. This binding stabilizes the biopolymer network which has been shown to improve formation of flocs and settling characteristics (Higgins and Novak 1997). The impact of bound extracellular proteins is especially interesting for granular sludge, since studies have shown that granular sludge has a higher protein to polysaccharide ration (PN/PS) when compared to activated sludge (McSwain et al. 2005).

An additional theory that applies to sludge bioflocculation and dewatering is the double layer theory (DLVO-theory). This theory suggests that particle charge is created by a double layer of counter-ions found on the surface of the particle. This double layer provides an electric potential on particle surfaces (measured as the zeta-potential), which contribute to repulsion forces. A research study found that increased EPS led to an increase in protein content, which is associated with high negative surface charge, theoretically making it more electrostatically repulsive. However, Mekkelsen and Keiding 2002 found that EPS with high concentrations of proteins led to an increase in floc stability which ultimately improved filterability. Therefore, it was hypothesized that the floc stabilizing role of EPS had more of an effect on bioflocculation than increased zeta-potential (Mikkelsen and Keiding 2002).

EPS creates a network capturing microbial cells to produce compact structures with a greater liquid solid interface. However, the EPS matrix may bind excess amounts of bound water due to the structure and chemical composition of the EPS. Therefore, developing a method to

fundamentally understand the mechanisms by which EPS contributes to distinguish water forms in wastewater sludge is essential to predict the dewatering capability of granular sludge.

3.0 Methods and Procedures

3.1 Reactor Setup

Two pilot sequencing batch reactors (SBRs), see Figure 6, were operated at the Lawrence, Kansas River Wastewater Treatment Plant. Aerobic granular sludge was formed and sustained with primary effluent wastewater as the feed. The working volume of the reactors was 70-L with a volumetric exchange ratio (VER) of 50%. A 3-hr SBR cycle was implemented with the following regime: 40 minutes of anaerobic fill supplied upward through the settled sludge bed, 122 minutes of aerobic mixing, 8 minutes of settling, and 10 minutes for decanting. Mixing was completed with coarse bubble diffusers and dissolved oxygen concentrations supplemented via fine bubble diffusers. The pilot reactors were operating at steady state for approximately six months before testing for dewaterability commenced. The dissolved oxygen was controlled at 4-5 mgl/L of O_2, and controlled online via SCADA. The average MLSS was 3000 mg/l ± 400 mg/l. Target wasting was performed prior to and during the dewatering experiment to provide an external selector for maintenance of granular particles and wastage of fines. This was accomplished by sieving biomass through a 0.2 mm sieve to target an F/M ratio of approximately 0.2 g rbCOD/gVSS-d. Sludge residence time (SRT) after wasting averaged at 6.5 days ± 1.2 days. Effluent suspended solids were approximately 26 mg/l ± 7 mg/l. The average granule size at the start of experimentation was 0.54 mm.

Figure 6: Photo of pilot reactors located at the Kansas River Wastewater Treatment Plant

3.2 Wastewater Composition

The reactors were bottom fed with primary effluent wastewater to establish plug flow conditions. The chemical oxygen demand (COD) of the primary effluent wastewater ranged from 130-300 mg/L, with the readily biodegradable substrate (rbCOD) portion ranging from 15 to 104 mg/L. Nutrient concentrations ranged from 0.5-5 mg-P/L phosphate and 15-30 mg/L ammonia-N. The daily loading rate to the reactors ranged from 0.5-0.9 kg COD/m^3-d. The reactors were operating stably for six months prior to dewatering tests.

3.3 Batch Polymer Demand Test Design

Batch tests were used to determine the optimal polymer dose and subsequent % solids content after centrifuging, which was adapted from the *Higgins Dewatering Test* (Higgins et al.

2014). Granular/flocculant sludge and activated sludge were sampled on the day of testing. Granular sludge was sieved through a 0.3 mm sieve to remove any fines and select for granules. Activated sludge sampled from the Kansas River Wastewater Treatment plant located in Lawrence, KS was collected as a grab sample from the aeration basin. Phipps and Bird 2L square beakers were used with a volume of 500 mL added to each beaker. Solid concentrations were targeted at approximately 2% solids before testing. To achieve this, the sludge was settled for 30 minutes prior to testing. Polymer dosages were added to each beaker, and mixing at 1000 g for 30 seconds was initiated. After 30 seconds, the mixing intensity was decreased to a g-force of 100/s and was mixed for 90 seconds. An aliquot of each sludge sample with the polymer dose was tested by CST, with the fastest CST signifying the optimal polymer dose. The sludge injected with the optimal polymer dose was then gravity thickened over a section of Ashbrook Belt Filter, while the filtrate was collected for mass balance evaluation. The gravity thickened sludge was then centrifuged for ten minutes at 3000 g. The centrate was sampled and for further testing. The polymer dosages tested included 2, 4, 8, 16, 24, 30, and 40 pounds of polymer/ton of dry solids for Clarifloc N-6310 polymer supplied by SNF Polydyne (Riceboro, GA) at a concentration of 5 g/L. Mixed liquor suspended solids (MLSS) and volatile suspended solids (VSS) were tested for the centrate and filtrate for mass balance evaluation; along with % cake of the centrifuged sludge.

3.4 Phase 1: Method Development for Water Fraction Quantification

3.4.1 Sample Preparation

Thermogravimetric analysis was chosen to determine the water types within the sludge samples. Aerobic granular sludge, sampled from pilot reactors, and activated sludge, obtained

from the Kansas River Wastewater Treatment Plant, were utilized for this study. The pilot sludge was filtered through a 0.3 mm sieve to ensure removal of fines. Sampling was performed on the day of testing. After sieving, the granular sludge was settled for 30 minutes to achieve a % solids concentration between 1.5 - 3 % for a volume of 50 milliliters. The volume used for TGA analysis was kept constant at 80 µL, and the remaining volume of concentrated sludge was utilized for CST measurements. Activated sludge was also settled for 30 minutes prior to sample introduction to TGA and CST.

3.4.2 TGA Parameters and Analysis

The parameters and analysis for the TGA tests were adapted from Kopp and Dichtl 2000 (Kopp and Dichtl 2000) with additional modifications. TGA method development tests were performed with an SDT-Q600 thermal analyzer by TA Instruments. The TGA conditions included a 30 mL/min air flow rate supplied to the drying chamber, with temperatures ramped to their isothermal conditions. Quantification of the water types are derived from the curve of the drying rate plotted against moisture content (mass-water/mass-SS) of the sample. Different sample preparations for the mixed liquor suspended solids (MLSS) were employed, including MLSS grab samples, MLSS sludge bed settled for 30 min, and MLSS sludge centrifuged at 1000 rpm for 30 minutes. TGA tests were performed for both granular sludge (>0.3 mm) and activated sludge. Results were analyzed using R (https://www.r-project.org), and slopes of the drying rate versus mass of water per mass of solid were examined using the Students' T-test. Drying tests were performed with temperatures ranging from 25℃, 35℃, and 80℃ with test times ranging from 400-700 minutes depending on temperature. Different temperatures were tested to establish the optimal temperature for distinguishing water types in the samples.

3.4.3 Development of R-Code to Examine changes in Drying Rate

An analysis method to determine the different water types after TGA was needed to achieve standardization. Due to the nature of the large TGA data sets, R-programming (https://www.r-project.org) was chosen for documentation of analysis. The TGA produces data with outputs for each time increment of the test: mass of sample, change in weight with units of % change per minute, and other parameters not used for dewaterability analysis. The slopes of the drying rate versus mass of water per mass of solid were plotted. Distinct differences in slopes were observed during the duration of the drying test. Points of inflection were selected and examined using the Students' t-test for statistical difference between consecutive slopes. The slope and coefficient of determination was additionally found to ensure selection of appropriate sections. The code used for water fraction analysis is presented in Appendix A.

3.4.4 CST Test

The CST test was performed according to *Standard Methods for the Examination of Water and Wastewater (Federation and Association 2005)*. CST measurements were performed using a Triton instrument (Essex, England)type 304M CST with Whatman No. 17 filter paper. 6-8 mL of the prepared sludge samples were poured into the steel collar of the Triton instrument. Samples were previously concentrated to achieve a % solids of approximately 1.5 - 3%, and granular sludge was sieved, prior to testing, through a 0.3 mm sieve to remove fines. The 18-cm collar, utilized for fast settling particles, was chosen for CST measurements. Triplicates of samples were run for each CST test, and % solids were also measured. The final results are reported in time required for the water to move from one electric node to another in seconds,

divided by % solids. Normalization is advised by Standard Methods, due to the effect of solid concentration variation on CST measurements (Federation and Association 2005)

3.5 Phase 2: Effect of Sludge particle size, EPS and Cations on Water Forms

3.5.1 Experimental design

As mentioned above, EPS is an essential component of granule formation and stability. Studies indicate that granular sludge is composed of higher EPS content than activated sludge, therefore, this impact was further examined with regards to dewaterability during phase 2 of experimentation. One of the objectives for this experiment was to investigate the effect of EPS composition and structure on water fractions in granular sludge. This was achieved by weekly sampling of granules (sieved through 0.3 mm screen) from June to October 2017. During this period, the food to microorganisms (F/M) ratio was varied to allow the EPS content and structure to change in the sludge as previous work showed EPS was correlated with F/M ((Faraj et al. 2017), see Figure 7. The F/M ratio was applied to the reactor within the range of 0.10 to 0.23 g rbCOD/ gVSS-d by adjusting the daily sludge wasting. Experiments performed to test this included

- Studying the effect of EPS on granule dewaterability.
- Identifying the water fractions for flocculent sludge and granular sludge obtained from the same reactor.
- Investigating the impact granular particle size had on free, interstitial, surface and bound water fractions.

Extractions of EPS and alginate were performed for all tests and samples. In addition, the following tests were performed: solids measurements, image analysis and water fraction testing using TGA, and filterability measurements using CST tests.

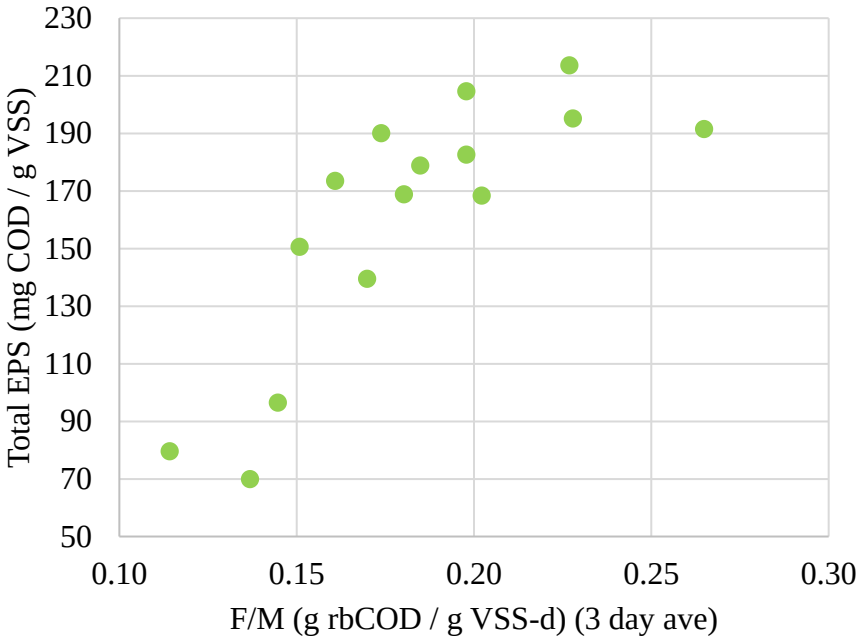

Figure 7: Effect of F/M ratio, an operational parameter, on Total-EPS production for granular sludge taken from (Faraj et al. 2017)

3.5.2 EPS and Alginate Extraction

Two methods for EPS extraction were used during dewaterability experiments: (1) the heat extraction method at 60℃ and (2) a heat and base extraction at 80℃ with sodium bicarbonate. The high temperature sodium bicarbonate extraction was utilized to further extract for alginate-like extracellular polymers (ALE). These two EPS extraction methods were performed to compare the total EPS extracted, and each is outlined further below.

The heat extraction method at 60℃ was adapted from Li and Yang 2007 to characterize the LB-EPS fraction. Approximately 0.15 g of VSS was resuspended in 30 mL of heated molecular grade Milli-Q (MQ) water to 60^0 C and vortexed for 1 min at 3000 rpm. Afterwards, the sample was centrifuged at 12,000 g for ten minutes, and the organic matter in the supernatant was collected as the LB-EPS fraction for further testing.

The method used to extract EPS using 80℃ and sodium bicarbonate was adapted from (Felz et al. 2016) and targeted Total EPS including alginate like extracellular polymers (ALE). Water was preheated to 80℃. 0.35 grams of VSS was suspended with 50 mL of MQ water. Sodium bicarbonate was added to the flask to obtain a 0.5% (w/v) sodium bicarbonate concentration. The flask was then placed in a water bath and covered with foil to prevent evaporation. The mixture was stirred for 35 minutes at 400 rpm at a temperature of 80℃. The solution was then centrifuged at 4000 g at 4℃ for 20 minutes. The centrifuged supernatant was dialyzed for 24 hours in a dialysis bag supplied by Fisher Brand with 3,500 Da molecular weight cut off to obtain the total EPS. After the dialysis, the extracted EPS was acidified to a pH of 2.2 to separate ALE products. After acidification, the product was centrifuged at 4000 g for 20 min and the resultant pellet represented ALE in the acid form. Finally, the pH of the pellet was adjusted to pH 8.5 to obtain ALE in the salt form for further testing.

3.5.3 EPS Characterization

The extracted total EPS, LB-EPS, and TB-EPS were analyzed further for COD, proteins and carbohydrates. Hach 21259-25 COD Test Kits were used for COD analysis, and these were digested using a Hach dual block DRB 200 reactor. Proteins were measured according to the Lowry Assay with Bovine serum albumin as the standard (Lowry et al. 1951). The Lowry

solution was freshly prepared on the day of the measurement. A sample volume of 0.2 milliliters was added to 10 milliliter glass tubes and 1 milliliters of Lowry reagent was added to each tube, with a v/v ratio of 100:1:1 for the alkaline solution composed of sodium hydroxide and sodium bicarbonate, copper sulfate and sodium tartrate respectively. The sample mixture was vortexed and then incubated in the dark for 10 minutes. After incubation, 0.1 milliliters of 2 N folin reagent diluted 1:1 with MQ water was added to each sample tube and vortexed. The samples are incubated once more for 30 minutes. Once incubation was complete, the samples were vortexed, and 0.2 milliliters of sample mixture was pipetted into a 96-well plate counter and read at an absorbance of 750 nm. All samples were performed in duplicate.

Carbohydrates were quantified utilizing the Dubois method (DuBois et al. 1956). Dextrose D-glucose was used as a standard for this method. Carbohydrates analysis was performed following EPS and alginate extractions. 400 microliters of sample was added to glass COD vails, with ten microliters of 80% (w/w) phenol and 1 milliliter of concentrated certified ACS-Plus sulfuric acid (Fisher cat. # A300212) also added to the sample tubes. Samples were vortexed and digested for 5 minutes at 90°C. After digestion, samples were placed in a dark room to cool to room temperature for 30 minutes. The absorbance was read at 490 nm using a 96-well count plate reader. Carbohydrates were also tested for the extracted alginate samples. A sodium alginate standard was used instead of glucose, and absorbance was read at 480 nm.

3.5.4 Solids Measurements and Image Analysis

During experimentation, MLSS and VSS were collected for each sample in duplicate according to *Standard Methods for the Examination of Water and Wastewater* (Federation and Association 2005). Characterization of particle size was performed using a stereomaster

microscope from Fisher Scientific once a week during testing. ImageJ software (https://imagej.nih.gov/ij/) was used for quantification of particle sizes during experimentation. Parameters measured included mean particle diameter and aspect ratio.

4.0 Results and Discussion

4.1 Phase 1: Method Development for Determination of Different Water Types

4.1.1 Temperature Comparison

To accurately quantify the different drying rates for water fractions within wastewater sludge, varying temperatures utilized for TGA were tested to ensure optimal visualization. Samples for granular sludge were taken from pilot SBR reactors operating at the Lawrence Kansas WWTP fed with primary effluent, and activated sludge was sampled from aerobic basins at the same WWTP also fed with primary effluent. Selected test temperatures were based on Kopp and Dichtl 2000 and Deng et al. 2011, which tested drying temperatures of 35 ℃ and 80 ℃, respectively (Kopp and Dichtl 2001, Deng et al. 2011). A lower temperature of 25 ℃ was also tried to see if visualization could be improved. Drying curves have been used in studies to observe the changes in drying rates, which are attributed to changes in removal of different water fractions. TGA tests were done with humidity kept constant by a supplying an air flow of 30 mL/min to the drying chamber, and ramp rates for each temperature were also maintained at 1 ℃/min for each TGA test. Once temperatures were raised to their target level, an isothermal condition was established for the duration of the test.

For all temperatures, there was evidence that four drying rates existed for both activated and granular sludge. This can be seen in Figures 8 (25℃), 9 (35℃), and 10 (80℃) as different slopes are visually distinguished. This factor is crucial for determining water fractions and will be expanded upon in the discussion regarding different water fractions and the effects on filterability. Free water is understood to be the first water type to be removed during drying tests, and is shown as the near constant slope beginning at the highest drying rate. The change from

free to interstitial water is observed when the first transition from the slope of the free water drying rate occurs, and is followed by the drying rate of surface water. Bound water is the steep decline in drying rate to an evaporation flux of zero, and begins when the slope of surface water transitions sharply. All of these slopes are identified visually, however, statistical analysis was performed between slopes to ensure selection of slopes was statistically significant. The test temperatures of 25 ℃ and 35 ℃ provided similar visualization of free and interstitial water drying rates, however, the rate attributed to surface water drying visually improved at a decreased temperature of 25 ℃ for both activated sludge and granular sludge. TGA analysis for granular sludge at 80℃ was not performed.

Analysis of the data sets were accomplished by plotting gram water per gram solid (Mw/Ms) in dependence to the rate of % water removed per minute (%/min). Mw/Ms was calculated using Equation 1 shown below, and percentage of water existing as free, interstitial, surface and bound was calculated using Equation 2. Transitions between slopes were selected using the R-code described in Appendix-A, and selected transition points were matched with the percent of free, interstitial, surface and bound water remaining during the drying test, and the gram of water per gram solid (Mw/Ms) data. Results are reported in terms of % water for each water fraction and Mw/Ms in g/g. These were found by subtracting the transitions corresponding with each water fractions, i.e. percentage of free water was found by subtracting the % of free water at the start of the test minus the percentage at the transition from free to interstitial water.

$$\frac{Total\ Weight - Solid\ Weight}{Solid\ Weight} \qquad Eq.1$$

$$\frac{(Total\ Weight - Solid\ Weight)\ for\ n-time\ of\ testing}{(Total\ Weight - Solid\ Weight) - during\ start\ of\ test}\ X\ 100 \qquad Eq.2$$

Since the scope of this research was to develop a method to accurately determine the different water contents within granular sludge, the test temperature of 25 ℃ was selected for further analysis due to the improved distinction between interstitial and surface water, which proved more difficult at higher temperatures. It is important to note that this decrease in temperature resulted in a longer testing time. The testing time for the 80℃, 35℃, and 25℃ TGA runs increased from approximately 90 minutes, 400 minutes and longer than 700 minutes for each respective temperature. Curves below are presented for granular and activated sludge at the varying target temperatures.

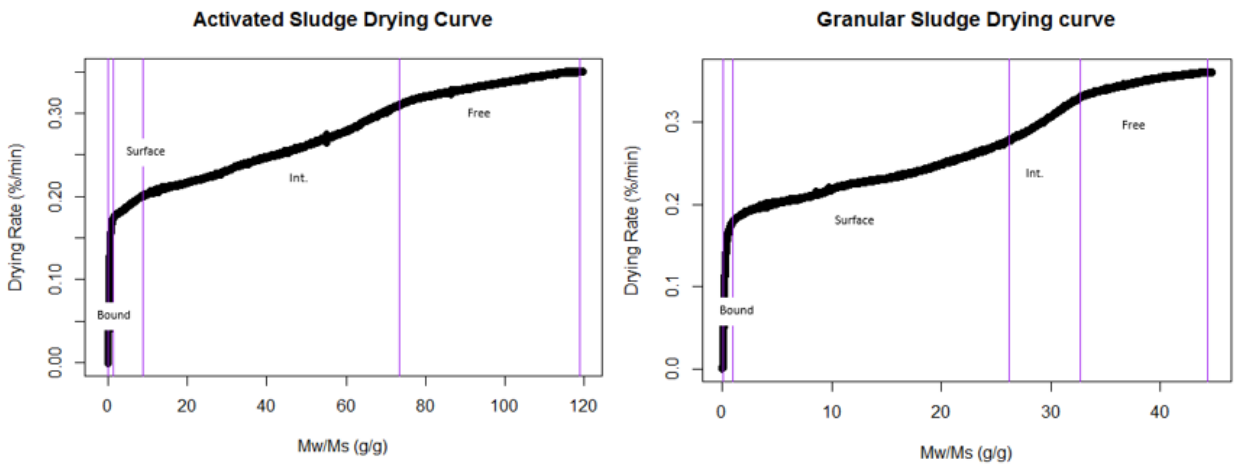

Figure 8: TGA curves for activated sludge and granular sludge at 25℃

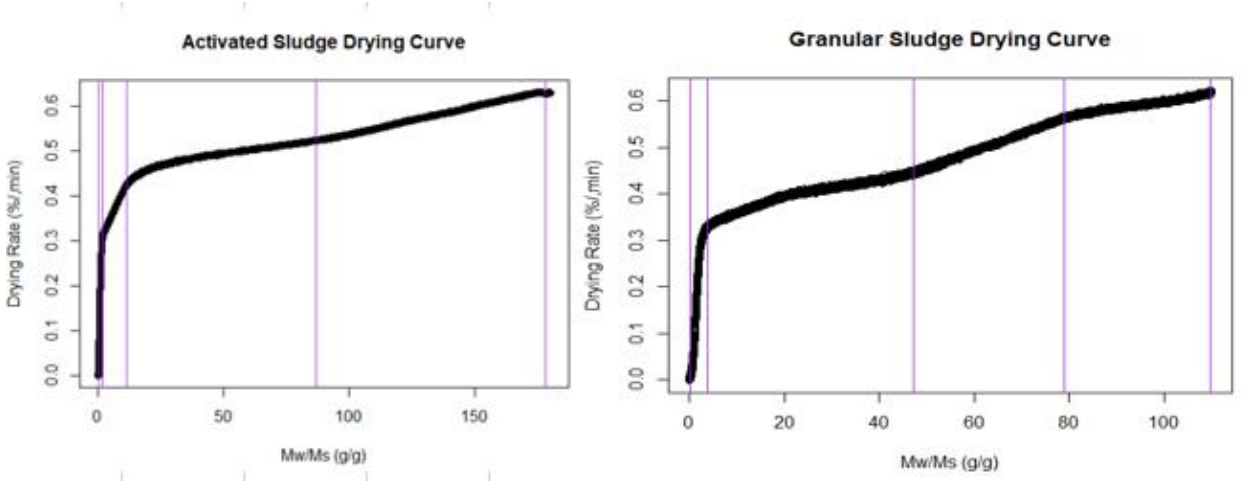

Figure 9: TGA curves for activated sludge and granular sludge at 35℃

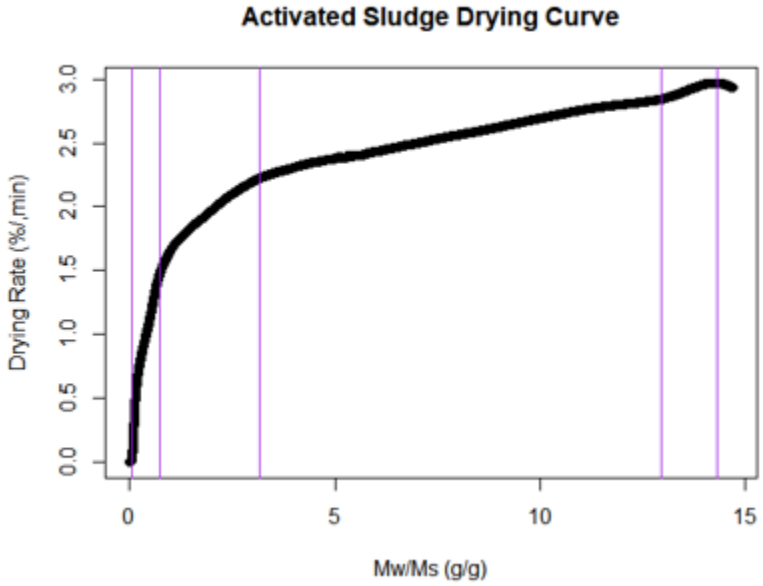

Figure 10: TGA curve for activated sludge at 80℃

To ensure all water was evaporated from the sample after TGA analysis was conducted at 25℃, the sludge pellet collected from the TGA drying chamber was placed in a 105℃ oven for more than one hour. The weights before and after drying in the 105℃ oven was recorded to see

if any decrease in weight was exhibited. There was no change in weight, so it was concluded that all water was evaporated during TGA testing.

4.1.2 Sample Preparation Comparison

Sample Preparation was examined to perceive the differences in drying curves and water composition for activated sludge obtained from aeriation basins at the Kansas River Wastewater Treatment Plant, and granular sludge sampled from pilot reactors fed with the same primary effluent. Sample preparation methods included: MLSS grab samples, 30 minutes of sludge settling, and sludge centrifuged at 1000 g for 30 minutes. Table 1 lists the percentage of water for free, interstitial, surface and bound; as well as the moisture content in g-water/g-solid for each respective group.

Table 1: Water Content in Granular and Activated Sludge with Different Sample Preparation *AS-Activated Sludge, GS-Granular Sludge

Sample prep.	Unit	Sample Type	Free	Interstitial	Surface	Bound
Unsettled Raw Sludge	% Water	AS	50.6	42.0	6.4	1.0
		GS	33.7	26.1	36.8	3.3
	Mw/Ms (g/g)	AS	106.9	79.5	16.3	2.6
		GS	35.5	37.8	54.2	4.8
Settled Raw Sludge	% Water	AS	37.0	53.7	8.1	1.1
		GS	30.9	14.8	52.0	2.4
	Mw/Ms (g/g)	AS	43.3	68.2	10.0	1.4
		GS	11.4	7.0	24.7	1.5
Centrifuged Raw Sludge	% Water	AS	25.1	22.6	51.1	1.1
		GS	24.2	14.7	56.6	4.6
	Mw/Ms (g/g)	AS	11.6	19.2	43.3	0.9
		GS	1.89	2.53	9.76	0.8

It is evident with all sample preparation that activated sludge contained a greater moisture content with respect to free and interstitial water; however, granular sludge had a larger moisture content for both surface and bound water. Activated sludge contained approximately 50% free water in the unsettled grab sample, while granular sludge grab samples had 34% free water. Settling for thirty minutes removed both free and interstitial water in both activated and granular sludge samples. However, activated sludge still had more free and interstitial water remaining than granular sludge. After settling, activated sludge had 37% free water and 53.7% interstitial water remaining, while granular sludge had 30% free and 14.8% interstitial water remaining. TGA drying curves are shown below for each sample preparation for both granular sludge and activated sludge, see Figure 11, 12, and 13 below. The sludge samples that were centrifuged exposed an interesting relationship with surface water. More than 50% of the water remaining after centrifuging for both activated sludge and granular sludge was surface water. This is may be caused by the physical act of compressing sludge particles together during centrifuging, which aids with increasing the surface area of the sludge sample introduced to the TGA.

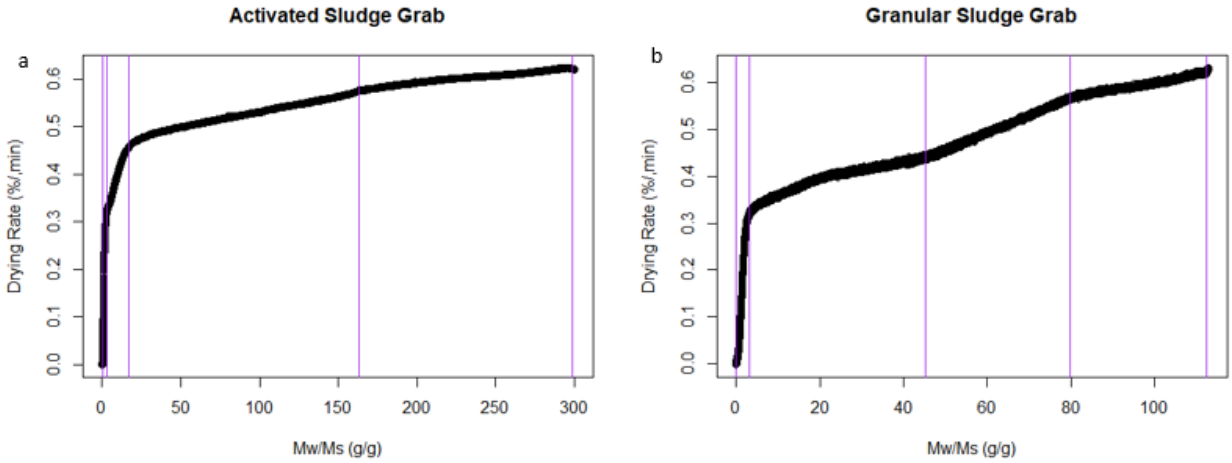

Figure 11: Drying curves for activated and granular sludge grab samples

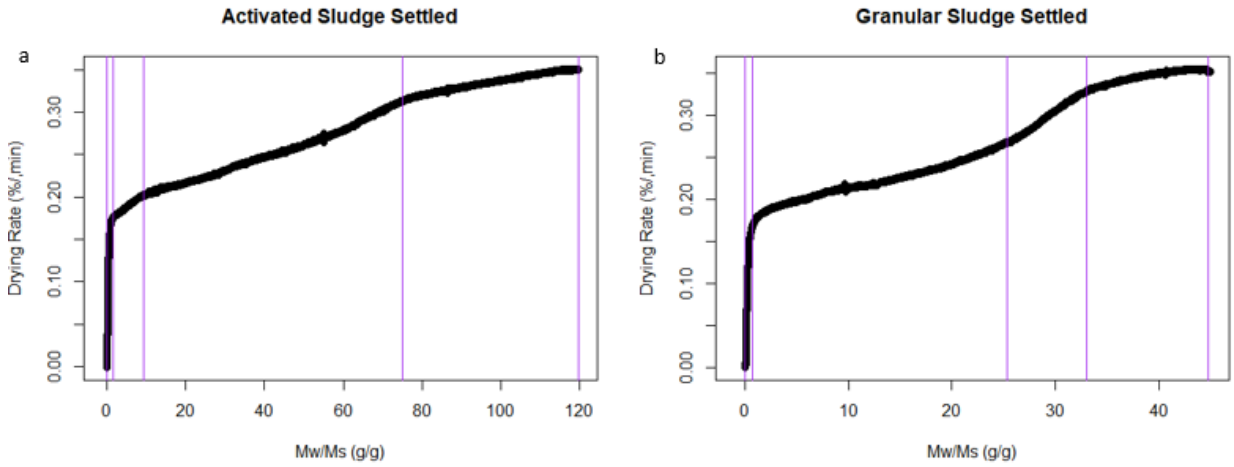

Figure 12: Drying curves for activated sludge and granular sludge after 30 minutes of settling

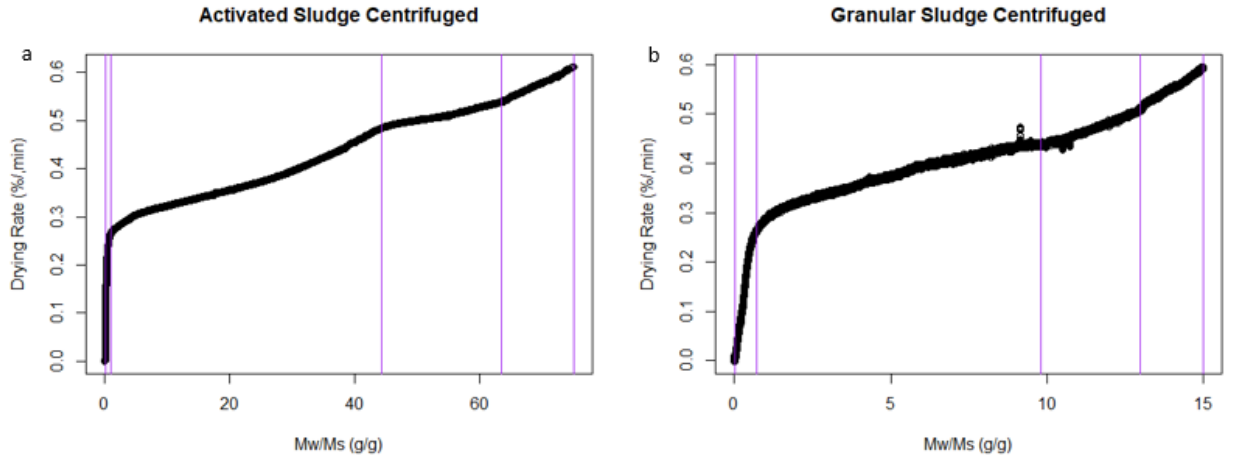

Figure 13: Drying curves for activated and granular sludge after centrifuging at 1000 rpm for 30 minutes

Past studies have revealed that activated sludge, or flocculent sludge, contains less EPS on average than granular sludge, with approximately 50% less proteins (McSwain et al. 2005). This greater abundance of EPS and proteins in granular sludge enhances the liquid solid separation (Urbain et al. 1993). An increase in extracellular proteins has also been shown to enhance

flocculating ability with belief that these proteins improve the attachment and binding of cells in a lectin-like manner (Higgins and Novak 1997), improving the hydrophobicity of the overall sludge (Jin et al. 2004).

Not all EPS provides aid for dewatering, and many researchers argue that properties and composition are more significant than total amount in terms of flocculation (Liao et al. 2001). An abundance of loosely bound EPS (LB-EPS) may lead to an increase of water trapped in crevices and pores. One study indicated that an abundance of LB-EPS resulted in a deterioration with bioflocculaton and filterability (Novak et al. 2003, Li and Yang 2007). An excess amount of LB-EPS changes the morphology of the sludge particles and allows for increased porosity within the sludge structure (Faraj 2014). Studies also suggest that high LB-EPS led to a weakened floc structure and decreases in cell attachment (Yang and Li 2009). This increase in crevices and pores allows for more areas in which interstitial water can become trapped. Activated sludge contained approximately 42% interstitial water from the grab sample, while granular sludge consisted of 26% interstitial water.

Interestingly, these results suggest that centrifuging activated sludge removes some of the interstitial water, which is in line with research suggesting that mechanical dewatering units squeeze interstitial water out of the pores and crevices during dewatering (Wu et al. 1998). Yet, this was not observed with granular sludge, which had little change in interstitial water. After settling for thirty minutes, the solids content of granular sludge is approximately 1-3% solids, and activated sludge was 0.6-0.8% solids. Studies have found that high solids content can physically squeeze out water trapped between flocs (Katsiris and Kouzeli-Katsiri 1987, Robinson and Knocke 1992, Smith and Vesilind 1995) and may be the reason behind granular sludge having less interstitial water. Thus, mechanical dewatering is less controlled by the interstitial portion of

granular sludge. However, other studies propose a theory that only free water can be removed mechanically (Kopp and Dichtl 2001).

While many agree that greater removal of free water coincides with better dewaterability, the results obtained from TGA analysis point to granular sludge retaining high amounts of surface and bound water after all preparation methods. This may be linked to an increase in EPS, gel-like by nature, capturing and retaining excess water due to the binding of water to the macromolecules, such as proteins, to its structure (Chen et al. 2001). There are two types of water binding mechanisms which take place with EPS and water. These include hydrogen bonds between the functional group of the EPS and the water dipole, and electrostatic interactions between the hydroxyl groups of EPS with water molecules (Neyens et al. 2004, Sheng et al. 2010). Therefore, it can be presumed that a higher concentration of total EPS may result in increased binding with water molecules, which is shown with granular sludge.

The results detailed in Table 1 suggest that mostly free and some interstitial water are expected to be removed during full-scale dewatering processes and that granular sludge will retain more bound and surface water in its structure than activated sludge during dewatering. However, the efficiency of removal of interstitial water for dewatering activated sludge is variable, and there may be less need for polymer to aid dewatering of granular sludge, since less free and interstitial water remain after gravity thickening. This will be further expanded in Section 4.1.5: Optimal Polymer Does and Resultant % Cake.

4.1.3 Water Distribution for Flocculent vs. Granular sludge Sampled from Pilot Reactors

Differences in granular sludge and flocculent sludge were similarly examined by performing tests with both flocculent sludge and granular sludge obtained from the same reactor. The method used to establish flocculent sludge and granular sludge was sieving the sludge sampled from the pilot reactor through a sieve size of 0.3 mm pore diameter. The underflow was used and tested as flocculent sludge, while the sludge captured on the sieve (>0.3mm) was considered granular (Adav et al. 2008). These tests were performed in consecutive days to ensure sludge composition was relatively similar between testing, and prepared by gravity settling for 30 minutes prior to TGA analysis.

Flocculent sludge, after gravity settling, contained 40% free water, and granular sludge contained 33.5% free water. Flocculent sludge also contained more interstitial water, with 32.2%, and granular sludge having 24.8% interstitial water. As observed previously with the preparation comparison, granular sludge had increased surface and bound water, with 39% and 2.6%, compared to activated sludge with 25.8% and 1.1%, respectively. See Table 2 for details regarding the values from the floc vs. granule comparison, and Figure 14 for visualization of flocculent and granular sludge drying curves. Table 3 displays the slope and coefficient of determination for each curve associated with free, interstitial, surface and bound water fractions.

Table 2: Comparison of Flocculent Sludge and granular sludge water fractions obtained from the same reactor

Sample Origin	Sample Type		Unit	Free	Interstitial	Surface	Bound
Pilot SBR Reactor	Flocculent (<0.3 mm)	=	% Water	45.3	29.2	24.5	1.1
	Granular (>0.3 mm)		% Water	30.3	26	41.5	2.1
Sample Origin	**Sample Type**		**Unit**	**Free**	**Interstitial**	**Surface**	**Bound**
Pilot SBR Reactor	Flocculent (<0.3 mm)	=	Mw/Ms (g/g)	32.8	22.6	18.9	0.8
	Granular (>0.3 mm)		Mw/Ms (g/g)	12.5	11.5	18.4	0.9

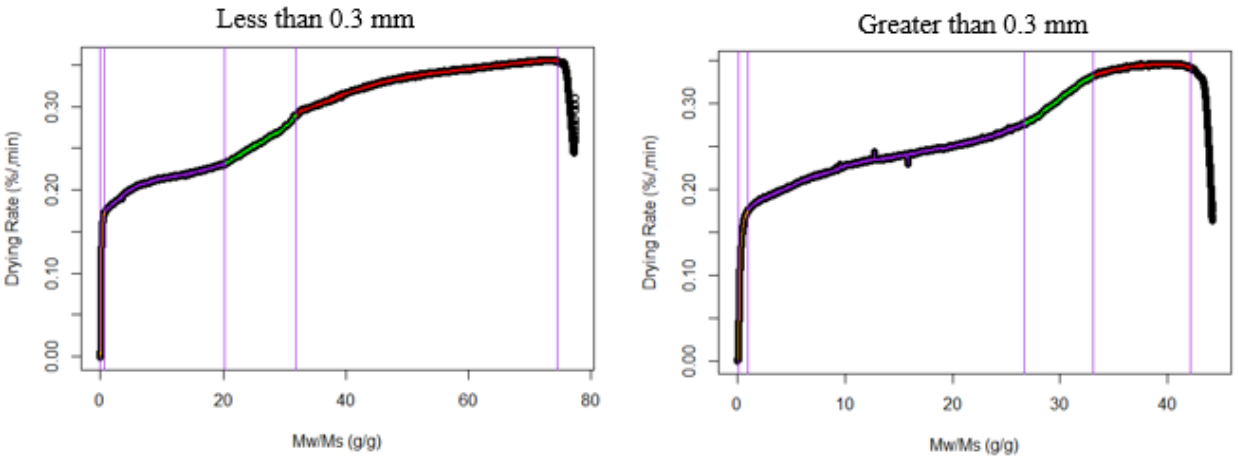

Figure 14: a) Flocculent Sludge Drying Curve and b) Granular sludge drying curve from same reactor: Red Slope = Free, Green Slope = Interstitial, Purple Slope = Surface, Orange Slope = Bound

Table 3: Slope and Coefficient of Correlation values for flocculent and granular Sludge based on Water Types

Sample Type	Unit	Free	Interstitial	Surface	Bound
Flocculent (<0.3 mm)	Slope	0.0010	0.0044	0.0025	0.2549
	R Squared	0.96	0.99	0.92	0.79
Granular (>0.3 mm)	Slope	0.0016	0.0053	0.0037	0.23
	R Squared	0.45	0.95	0.96	0.85

4.1.4 Capillary Suction Time Comparison between Activated Sludge and Granular Sludge

Capillary suction time is a commonly used test to obtain the filterability of a sludge or biosolids sample. There have been no reports of CST utilized for granular sludge; therefore, a baseline to compare with literature was not available. Instead, granular sludge CST values were compared to activated sludge samples with similar solids concentrations. It is important to note that CST tests are affected by solids concentrations; therefore, the final CST values were normalized with % solids resulting in a unit of seconds/ % solids.

Table 4: CST results for activated sludge and granular sludge tested at different solid concentrations. CST is highly variable with solids concentration, therefore it is normalized with % solids content

CST Test for Activated Sludge			
Sample	CST (s)	% Solids	CST (sec /Solids)
1	7.6±1.2	0.09	84
2	9.7±0.9	1.24	7.8
3	16.1±1.4	2.9	5.6
4	16.3±1.3	3	4
CST Test for Granular Sludge			
Sample	CST (s)	% Solids	CST (sec /Solids)
1	7.1±1.35	0.3	24
2	7.7±1.4	1.6	4.8
3	10.8±2.9	3.6	3.0
4	15.0±0.7	4	3.8

Shown in Table 4, when the solids concentration increased, the CST showed a decreasing trend for activated sludge and granular sludge, which has been established in past studies (Vesilind 1988). After normalizing, granular sludge had faster CST results than activated sludge, particularly with low solid concentrations. However, there was more standard deviations for the granular sludge samples with higher % solids. This may be attributed to granular sludge having denser particles than activated sludge (Winkler et al. 2013) and significantly higher settling velocities (Morgenroth et al. 1997). This may pose issues for the CST test when used with granular sludge, because the fast settling characteristics alter and enhance the physical barrier created by the sludge particles as the liquid filters. This was previously examined and stressed for fast settling particles in Chen 1995, which suggested stirring while the CST test was underway (Chen et al. 1995). However, no stirring was done during testing for this experiment.

The effect of different water fractions on the CST measurement was also examined. One study suggested that CST is strongly influenced by the free water content in a sludge sample (Jin et al. 2004), which was supported with results obtained during this experiment, see Figure 15. It was found that CST measurements increased as free water and interstitial water increased in granular sludge. However, CST showed a declining trend when surface and bound water increased. Past literature attempted to correlate bound water and CST; however, differences in bound water classification presumably affected the outcome of the CST measurements.

Chen et. al., 1996 defined bound water as water remaining in sludge after mechanical dewatering (Chen et al. 1996), however, more recent literature outlines interstitial, surface and bound water continuing to remain after mechanical dewatering (Kopp and Dichtl 2000, Kopp

and Dichtl 2001, Deng et al. 2011). Chen et. al., 1996 found that an increase in bound water caused a decrease in the effective porosity. This decrease in effective porosity resulted in an increased CST measurement. However, effective porosity may well be influenced primarily by interstitial water trapped between the pores and crevices. Deng et. al., 2011 found that after mechanical dewatering, more than 50% of the water weight was attributed to interstitial water. Therefore, the trend established by LB-EPS and its effect on the CST measurement is comparable to the conclusion Chen et. al., 1996 made. As LB-EPS increased, an increase in the CST measurement was observed, see Figure 16. Therefore, it may be argued that Chen et al., 1996 consideration of bound water may have instead been interstitial water trapped in the pores of LB-EPS.

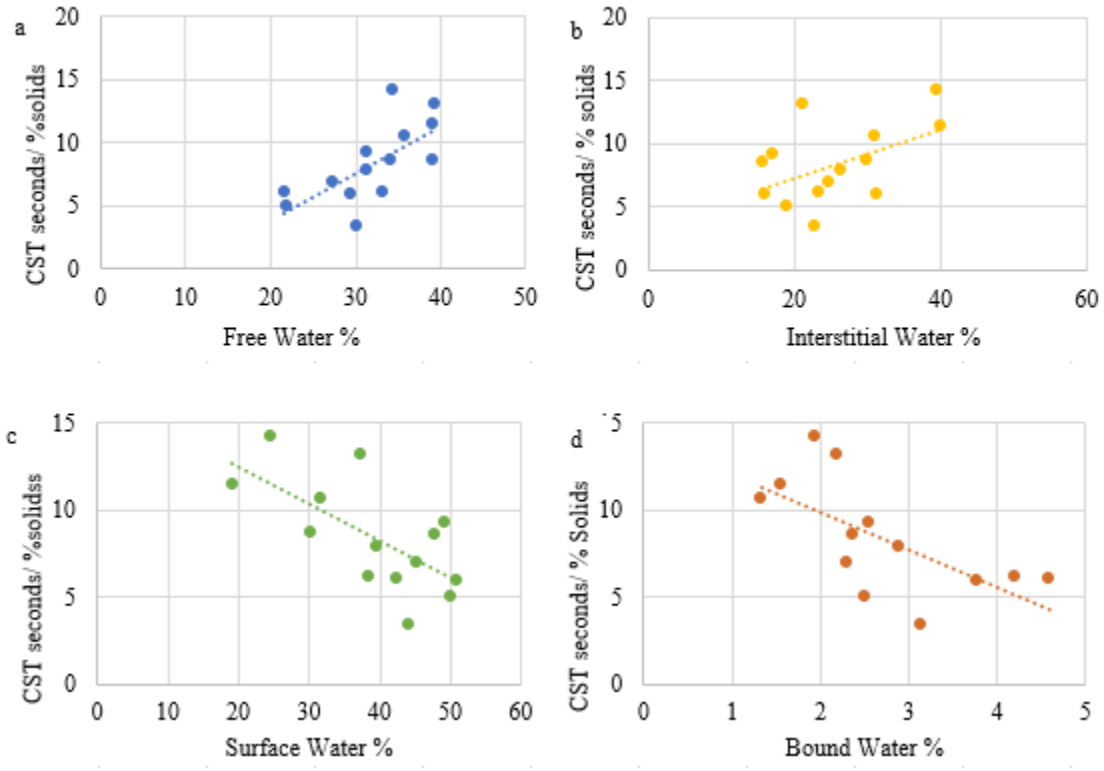

Figure 15: Impact of Free, Interstitial, surface and bound water on the CST measurement

Nevertheless, the correlation found between bound water and the CST measurement is still under question. Jin et al., 2004 found an increasing CST measurement with an increase in bound water, suggesting that high concentrations of bound water worsened filterability of wastewater sludge. Once again, bound water was classified as water remaining after mechanical dewatering, which was accomplished by centrifugation at 3000 rpm for 10 minutes. Kopp and Dichtl 2000 found that even after centrifuging wastewater sludge at 1000 g for 30 minutes, free, interstitial, surface, and bound water remained (Kopp and Dichtl 2000). This suggests bound water may not have been the only water fraction considered in the Jin et al., 2004 study, and may have affected the results of their CST measurement.

Total EPS, LB-EPS and proteins also exhibited impacts on the CST measurement for granular sludge. Figure 16 shows the relationships between these constituents. An increase in total EPS and proteins exposed a decreasing trend with the CST measurement. These results mirrored previous research that correlated total EPS and proteins with the CST measurement. Jin et al. 2004 found that as total EPS and proteins increased, there was a decrease in the CST measurement. However, this test was for activated sludge dewaterability (Jin et al. 2004). Additional studies have also found that increases in SRT led to an increase in protein to carbohydrate ratio. This increasing ratio within EPS enhances hydrophobicity (Liao et al. 2001), which theoretically aids in the repulsion of water. Knocke 1986 found a decrease in CST after the mean cell residence time increased (Knocke and Zentkovich 1986), and another study confirmed these findings with improvements on filterability and dewatering observed after the SRT for a membrane bio-reactor (MBR) increased from 23 to 40 days (Al-Halbouni et al. 2008). Hence, the finding from this experiment which show a decrease in the CST measurement for

granular sludge associated with an increase in total EPS and proteins can be considered consistent to previous research associated with activated sludge.

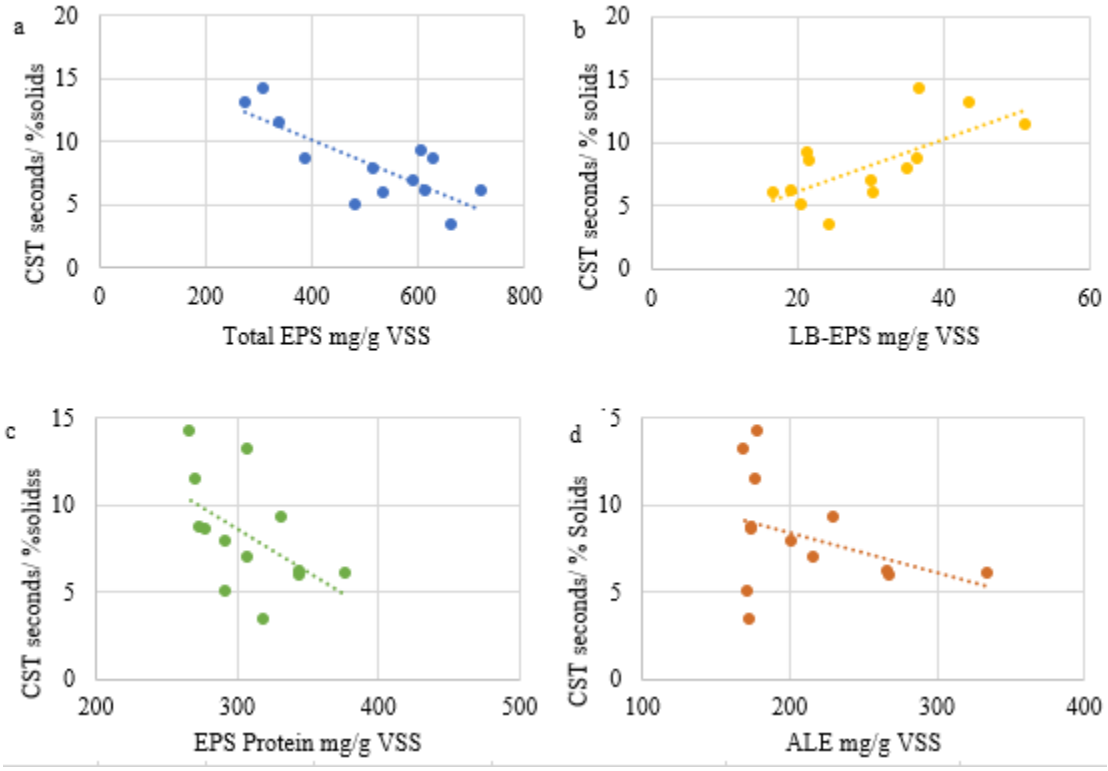

Figure 16: Effect of a) Total EPS b) LB-EPS c) Proteins in EPS and d) ALE on the CST measurement

The study performed by Al-Halbouni 2008 additionally discovered that low SRT's produced higher values of floc-bound EPS when compared to the MBR's operating at high SRT's (Al-Halbouni et al. 2008), which was also shown in a study conducted by Liu et al 2007 that found an increase in LB-EPS as SRT declined (Li and Yang 2007). In the 2008 study conducted by Al-Halbouni, the increase in the testing time for the CST measurement may be predominantly affected by the increase of pores and crevices associated with flocs containing high concentrations of LB-EPS. An increase in CST suggests a deterioration in dewatering

ability, while an increase in interstitial water also resulted in an increase in the CST measurement, see Figure 16; therefore, one can conclude that excess amount of LB-EPS may lead to entrapment of interstitial water, resulting in a deterioration of dewatering

4.1.5 Optimal Polymer Dose and Resultant % Cake

Although water content characterization is required to fundamentally understand the nature of granular sludge dewaterability, operators and wastewater treatment plant managers primarily care about the final percentage of cake for a treated biosolid and the polymer needed to obtain this value. Polymer dosages vary due to wastewater sludge characteristics and mechanical dewatering processes. Polymer is a substantial cost in terms of operations and requires optimal dosing to properly remove the highest percentage of water while remaining cost effective.

In order to establish the optimal polymer dose for granular sludge dewaterability, the Higgins Dewatering test, developed from (Higgins et al. 2014) was applied. Activated sludge was tested and compared to results obtained from full scale dewatering operations at the Lawrence Kansas River Wastewater Treatment Plant. Activated sludge required a polymer dosage of 32.4 lbs/ton to achieve the fastest CST test after conditioning, and granular sludge required a dose of 5.2 lbs/ton. After conditioning, sludge was centrifuged according to the Higgins dewatering test, and activated sludge had a cake of 21.7%, while granular sludge had a resultant cake of 24.9%. Polymer dosage preformed at the Lawrence Kansas River Wastewater Treatment Plant required an average of 36.6 lbs/ton of polymer, with an average % cake of 22.7% leaving the belt filter press, see Table 5. It is important to note that the values obtained from full-scale plant operations are after anaerobic digestion, thus, a deterioration in dewatering

may be observed due to the release of non-degradable polysaccharides which are freed during cell destruction (Kopp and Dichtl 1998, Novak et al. 2003). Hence, an increase in polymer for a comparable % cake is observed at full scale.

Table 5: Polymer dose and resultant % cake for granular sludge and activated sludge *Note full scale plant values for polymer dose and % cake is obtained after anaerobic digestion

Sample Type	polymer Dose	Polymer Type	% Cake	Cost per year
Activated Sludge	32.4 (lbs/ton)	Clarifloc	21.7	$30,000
Granular Sludge	5.2 (lbs/ton)	Clarifloc	24.9	$5,000
*Plant Values * After Digestion*	*36.6 (lbs/ton)*	*Clarifloc*	*22.7*	*$33,600*

When comparing granular sludge and activated sludge, there was a substantial decrease in polymer dosage for granular sludge, which would lead to a cost savings of more than $25,000 per year based on the current price of polymer used for plant operations at the Kansas Rive Wastewater Treatment Plant. From previous TGA results, it was found that granular sludge contained less free and interstitial water than activated sludge after gravity thickening, and literature explains that free and interstitial water are the factors controlling percent cake (Tsang and Vesilind 1990, Kopp and Dichtl 2001). Tsang et. al., 1990 stressed that free and interstitial water may remain after mechanical dewatering, and this could be considered as the inherent inefficiency of the selected dewatering processes (Tsang and Vesilind 1990). The previous findings of this research support the evidence that granular sludge required less polymer and produced a higher percent cake due to the decrease of both free and interstitial water, and the conclusion is also supported by CST measurements comparing granular sludge and activated sludge. However, dewatering granular sludge after anaerobic digestion needs to be examined to observe the possible deterioration due to cell destruction and release of unbiodegradable polymers and cations.

After performing the Higgins Dewatering Test for both activated sludge and granular sludge, samples before conditioning and after conditioning were examined using the TGA method described above. The results from the TGA analysis show that after conditioning, activated sludge had 33.2 % of the free water and 63.1% of the interstitial water removed. Conditioning resulted in the removal of 62% of the free and 36.7% of the interstitial water from granular sludge, see Table 6. Granular Sludge also showed a 5.4% removal of surface water, see Table 7.

Table 6: The % and mass (g) of free and interstitial water removed for activated sludge and granular sludge

Sample Type	Unit	Free	Interstitial
Activated Sludge	Water Removed (g)	5.5	11.0
	Water Removed (%)	33.2	63.1
Granular Sludge	Water Removed (g)	4.0	1.3
	Water Removed (%)	62.0	36.7

Table 7: % and mass (g) of surface water removed for granular sludge

Sample Type	Unit	Surface (g/g)
Granular Sludge	Water Removed (g)	0.2
	Water Removed (%)	5.4

Table 8: Free, interstitial, surface and bound water in activated sludge and granular sludge before and after conditioning

Sample Type	Prep.	Free (g/g)	Interstitial (g/g)	Surface (g/g)	Bound (g/g)
Activated Sludge	Before Cond.	16.6	17.5	1.8	0.6
Activated Sludge	After Cond.	11.1	6.5	4.2	3.5
Granular Sludge	Before Cond.	6.5	3.7	4.2	0.4
Granular Sludge	After Cond.	2.5	2.3	4.0	1.1

Interestingly, activated sludge showed an increase in both surface and bound water after conditioning, while granular sludge had an increase in bound water, see Table 9. For activated sludge the surface water increased from 1.8 g-water/g-solid to 4.2 g-water/g-solid. After conditioning, the bound water in activated sludge increased from 0.6 g-water/g-solid to 3.5 g-water/g-solid. The conditioning of granular sludge and its effects on bound water were similar for activated sludge, with bound water increasing from 0.4 g-water/g-solid to 1.1 g-water/g-solid for the granular sludge samples. This increase in surface and bound water for activated sludge can be theorized by the increase in surface area as the sludge particles coagulate. In terms of the specific polymer used, Clarifoc which is a cationic polymer, may bind and entrap surface water during flocculation. Additionally, bound water, also referred to as chemically bound water, may increase with the addition of a chemical coagulant, thus inducing the binding of excess water. Granular sludge required almost ten times less polymer than activated sludge, and this may be the reason why surface water remained relatively constant for granular sludge.

Table 9: % of bound water and surface water for activated sludge and granular sludge before and after conditioning with polymer * Note granular sludge showed no increase in surface water after addition of polymer

Bound Water (% of Total Water Weight)		
Sample Type	**Before Cond.**	**After Cond.**
Activated Sludge	1.6	14
Granular Sludge	2.6	12

Surface Water (% of Total Water Weight)		
Sample Type	**Before Cond.**	**After Cond.**
Activated Sludge	5	17

4.2 Phase 2: Effect of Sludge EPS and Particle Size on Water Forms

4.2.1 Effect of EPS on Water Fractions and Dewatering Potential

Experiments were carried out to determine the effect EPS had on water fractions and dewatering potential. Manipulation of EPS was done by controlling the F/M ratio in the granular sludge pilot reactors during experimental testing. CST tests were also performed for each sample.

The results in Figure 17 indicate that as total EPS in mg-COD/g-VSS increased, free and interstitial water decreased, while surface and bound water increased. As previously mentioned, the relationship between free water and the CST measurement signifies a positive trend, and free water is believed to not be bound to any surfaces or trapped between flocs. Additionally, granular sludge and biofilms have been confirmed as having more EPS then flocculent sludge (Martin-Cereceda et al. 2001, McSwain et al. 2005).This increase in total EPS improves bioflocculation and increases the negative surface charge of sludge particles, thus improving the hydrophobicity of the sludge particle and aiding in the liquid solid separation (Morgan et al.

1990, Liao et al. 2001). Therefore, it can be concluded that free and interstitial water should decrease as total EPS increases, and this is supported by data collected from this experiment. However, differences in EPS structure with regards to TB-EPS and LB-EPS have resulted in differences of distribution for water within the sludge samples.

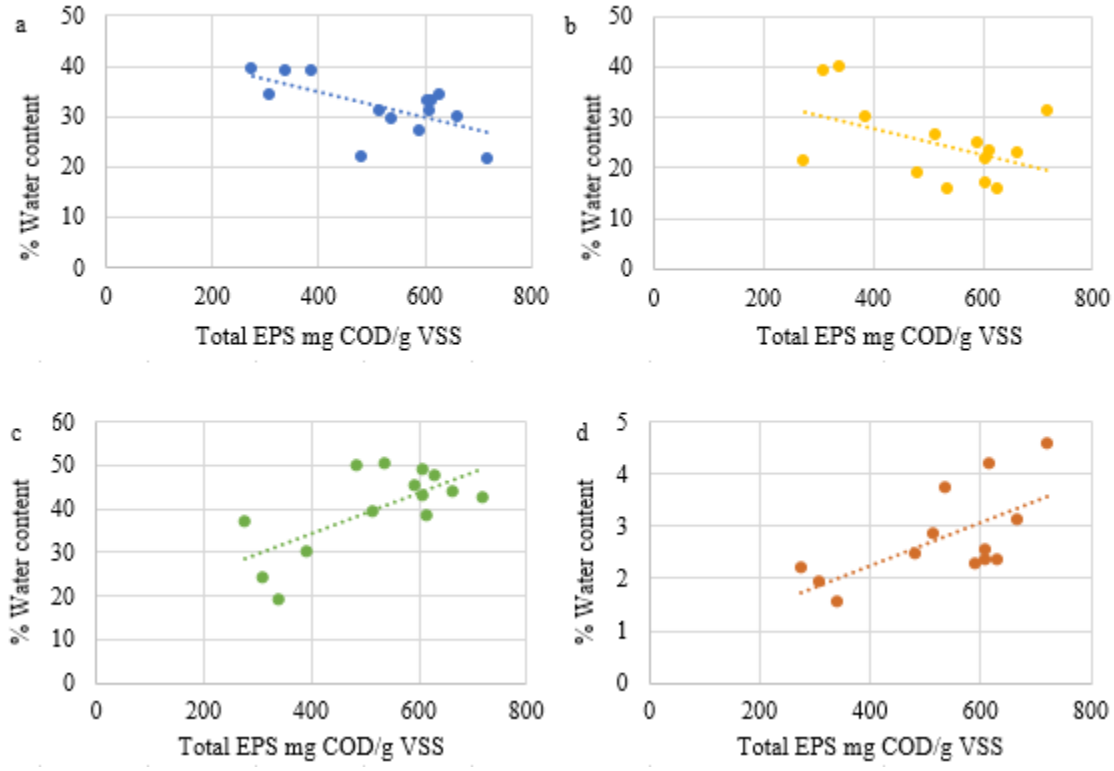

Figure 17: Impact of Total EPS on a) free water b) interstitial water c) surface water and d) bound water in granular sludge

Tightly bound EPS (TB-EPS) and Loosely Bound EPS (LB-EPS) showed linear and opposite trends with regards to water distribution. As TB-EPS increased, free and interstitial water decreased, with an increase in surface and bound water seen in granular sludge. An inverse relationship was found with LB-EPS, with an increase in free and interstitial, and a decrease in

surface and bound water, see Figure 18 and Figure 19 for relationships between TB-EPS and LB-EPS with regards to water types. Li and Yang 2007 discovered that as sludge residence time (SRT) was lengthened, LB-EPS concentrations decreased. This decrease was found to have a significant positive impact on sludge flocculation and separation (Li and Yang 2007). Li and Yang argue that the increase of LB-EPS results in a weakening of the floc structure. This weakened binding may possible allow for excess water to become entrapped within the loose EPS structure.

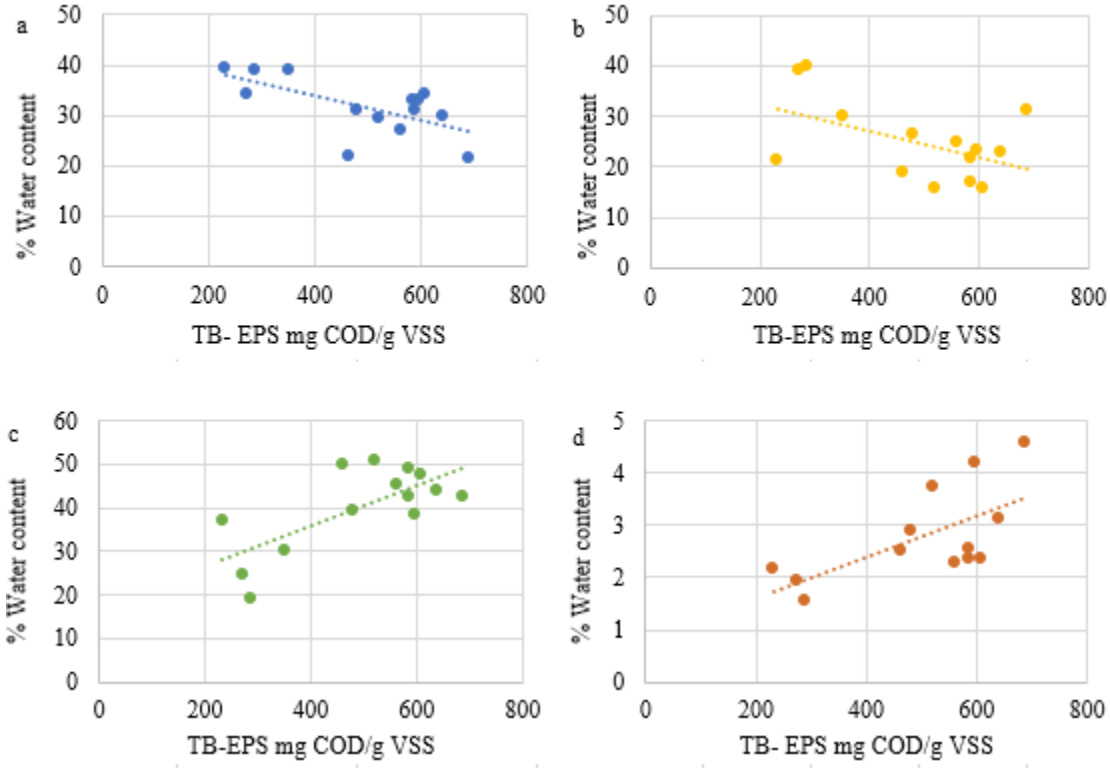

Figure 18: Effect of TB-EPS on a) free water b) interstitial water c) surface water and d) bound water in granular sludge

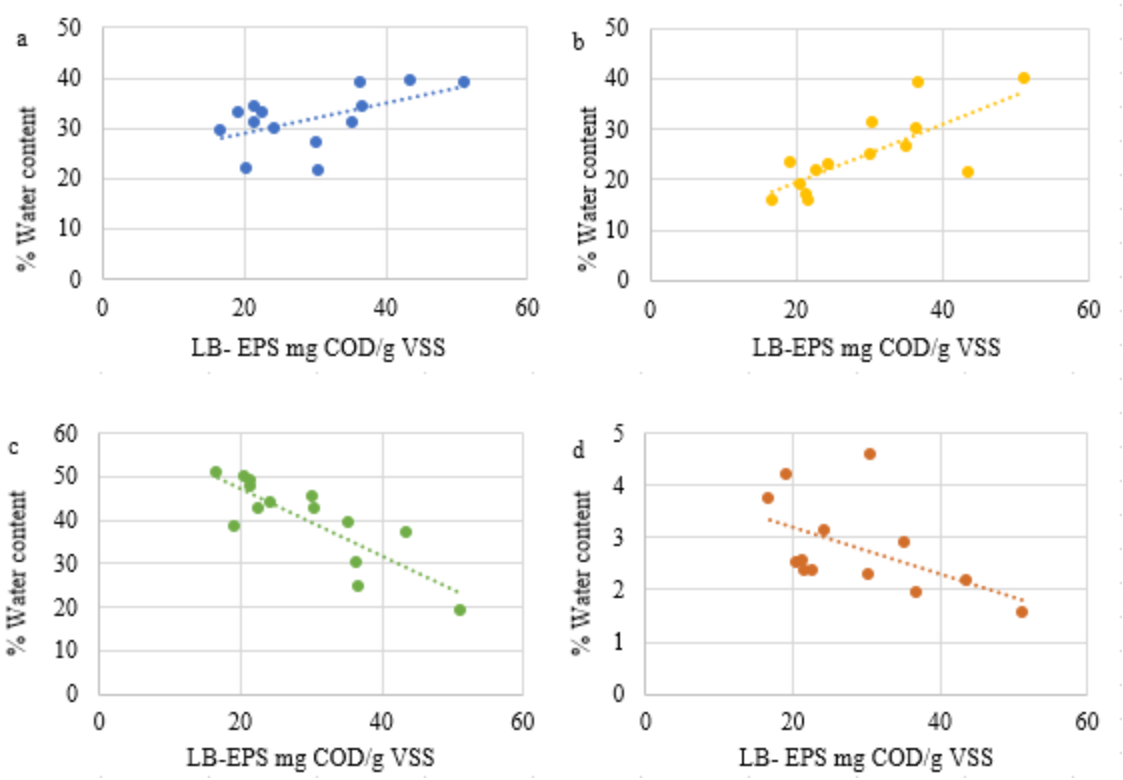

Figure 19: Impact of LB-EPS on the distribution of a) free water b) interstitial water c) surface water and d) bound water for granular sludge

Figure 20 shows the relationship between alginate-like exopolysaccharides (ALE) and water fractions. ALE influenced the percentage of free and bound water, but it exhibited no substantial trend with interstitial and surface water. However, the gel-like property of ALE should theoretically aid in the separation of free water, which is shown by the results in this experiment. ALE is also known to have an overall negative charge, which aids in repelling water (Lin et al. 2010). However, as ALE increased, bound water exhibited an increasing trend. Hydroxyl groups are present within the ALE structure, which will bind to water molecules with hydrogen bonding forces. Therefore, an increase in bound water may be resulting from this increase in hydrogen bonds. The correlation between interstitial and surface water with ALE is not as clear as free and bound water. In terms of surface water, there is an increasing trend which

may be attributed to the DLVO theory taking place by repulsion from hydroxyl groups bound to ALE, and the attraction of Van der Waals forces near the solid surfaces. It is shown below in Figure 20c, repulsion forces have a weaker effect on the percentage of surface water when correlated with ALE. Surface water may also be affected by other factors including the cations present within ALE, and the location of ALE. A deeper investigation may be needed to understand the relationship between surface water and ALE, and how cations and size may affect this.

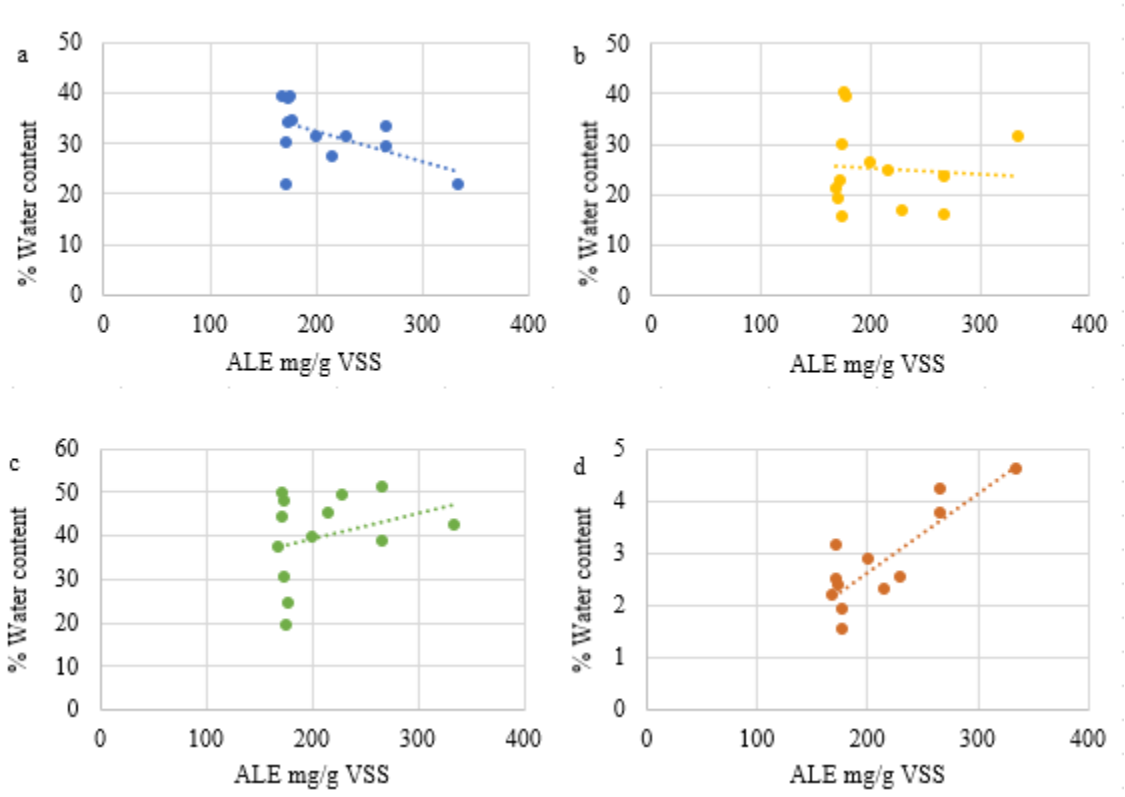

Figure 20: Effect of ALE on a) free water b) interstitial water c) surface water and d) bound water for granular sludge

Proteins within EPS were also shown to influence the free, interstitial, surface and bound water portion in granular sludge. An increase of proteins within the total EPS resulted in less free

and interstitial water for granular sludge, however, increased surface and bound water was observed, see Figure 21. Previous studies have found that an increase in protein content within EPS increases hydrophobicity of the sludge. It was also established that bacterial cells grown within biofilms had twice the hydrophobicity as cells in suspension (Martin-Cereceda et al. 2001, Liu and Fang 2003). Jin et. al., 2004 concluded that an increase in proteins within the extracted EPS led to a decrease in CST. This is supported by the results found for the impact proteins exhibited on free and interstitial water, see Figure 21. As proteins increase, free and interstitial water was shown to decrease, which is related to the decrease in the CST measurement. Similar to the effects of ALE with regards to bound water, water molecules can bind to the hydroxyl groups in the protein structure, hence, an increase in bound water is observed.

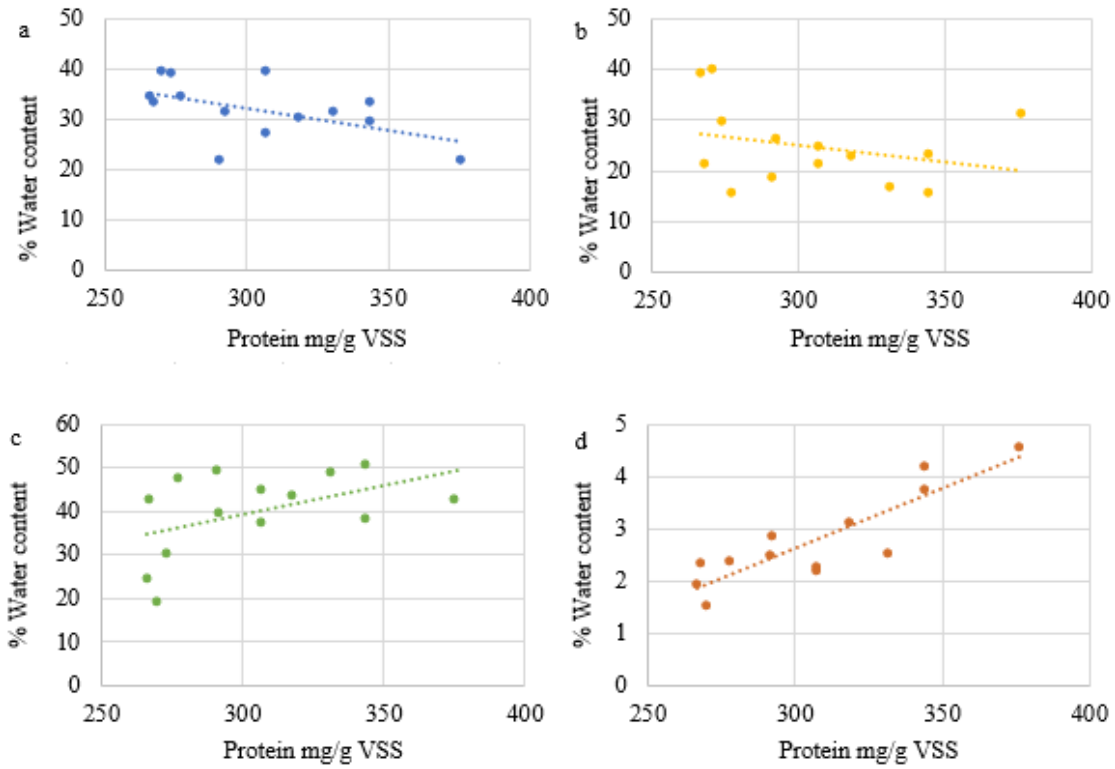

Figure 21: Impact of Proteins within the EPS on a) free water b) interstitial water c) surface water and d) bound water

A correlation matrix of EPS components and particle diameter, which will be discussed in the following section, can be found in Table 10. P-values are also displayed in the matrix to show statistical significance. Here it is detailed that proteins and ALE have the highest positive effect on bound water distribution within granular sludge; while total EPS and TB-EPS had the highest negative trend with regards to the CST measurement. LB-EPS exhibited an increasing and strong trend with interstitial water. Overall, these findings reveal how the structural differences in granular sludge aid and deter in filterability. TB-EPS is advantageous for reductions in free water. However, bound water increases as all components of EPS increase, except for LB-EPS. The role LB-EPS plays in water distribution is key with regards to increased interstitial water. Controlling the accumulation of LB-EPS by operational conditions such as F/M

ratio allow operators to possible decrease the amount of interstitial water to aid in dewatering of granular sludge. This will save costs with handling and disposal of dewatered sludges and biosolids.

Table 10: Correlation Matrix for EPS components and diameter of granules with each water Fraction and the CST measurement. Diameter and surface water had the highest correlation, followed closely by free water and the CST measurement

Component	%FW		%IW		%SW		%BW		CST (sec/%solid)	
	r	p	r	p	r	p	r	p	r	p
TB-EPS (mg COD/g VSS)	-0.633	0.015	-0.491	0.074	0.705	0.005	0.650	0.016	-0.793	0.001
LB-EPS (mg COD/g VSS)	0.542	0.045	0.754	0.002	-0.829	0.000	-0.518	0.070	0.686	0.010
Total EPS (mg COD/g VSS)	-0.627	0.016	-0.463	0.095	0.683	0.007	0.647	0.017	-0.785	0.001
Protein (mg BSA/g VSS)	-0.535	0.049	-0.287	0.319	0.474	0.087	0.868	0.000	-0.529	0.063
ALE (mg sodium alginate/g VSS)	-0.523	0.067	-0.066	0.831	0.308	0.305	0.848	0.000	-0.377	0.205
Diameter (mm)	-0.671	0.009	-0.666	0.009	0.820	0.000	0.495	0.086	-0.711	0.006

4.2.2 Impact of Particle Size on Water Distribution and CST Measurement

Analysis of TGA curves with respect to EPS components and granule particle size expose that particle diameter is the most important and strongly correlated factor in dewatering results. As shown in Table 10, the parameters with the one of the highest correlation belong to the relationship between diameter and surface water, see Figure 22.

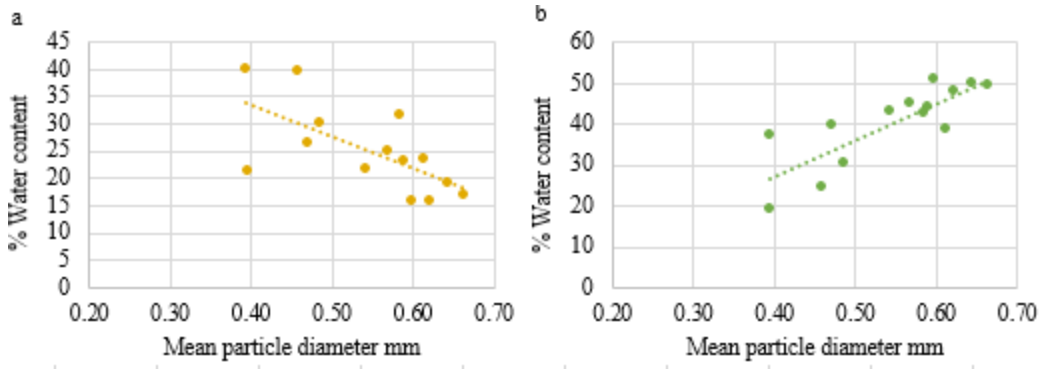

Figure 22: Influence of mean particle diameter on a) interstitial water and b) surface water

Particle size has long been considered an important factor in the dewaterability of wastewater sludge (Karr and Keinath 1978, Knocke and Zentkovich 1986). However, granular particle size distribution greatly varies from that of activated sludge, and the impacts of particle size have not been explored in terms of dewaterability.

A study conducted by Karr 1978 found that supercolloidal solids in the range from 1-100µm had the most negative influence on dewatering based on SRF and CST tests (Karr and Keinath 1978). Another study, Knocke et. al., 1986 found that an increase in SRT lead to increases in particle size, which improved filterability. Knocke concluded that as particles coagulate to form larger particles, a decrease in total drag force is accomplished by decreases in surface to volume ratios, and this decrease in drag is the main contributor to improved filterability. The study conducted by knock are in line with results obtained in this experiment. CST measurements showed to have a negative trend as particle size increased for granular sludge, see Figure 23. This decrease in CST is associated with improved filterability.

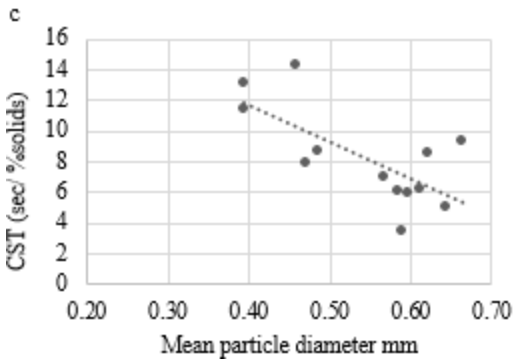

Figure 23: Impact of mean particle diameter on CST measurement

Possibly more intriguing, are the results found for mean particle diameter and its effect on surface water. As diameter of the particles increased a positive trend was observed for percentage of surface water, while a decreasing trend was revealed for interstitial water. Increased surface area will ultimately lead to increases in sites for solvation forces. However, this is not only on the outer core of the granule, but will also include water bound to the surface of cells. This theory is also supported with polymer dosage testing, which showed an increase in surface water for activated sludge that required substantially more polymer to effectively coagulate when compared to granular sludge. Figure 24 shows that granular sludge particles that are greater than one millimeter had the least amount of free water, and the highest concentration of surface and bound water, and the size fraction of less than 0.2 mm and between 0.2mm-0.4mm have the most free and interstitial water.

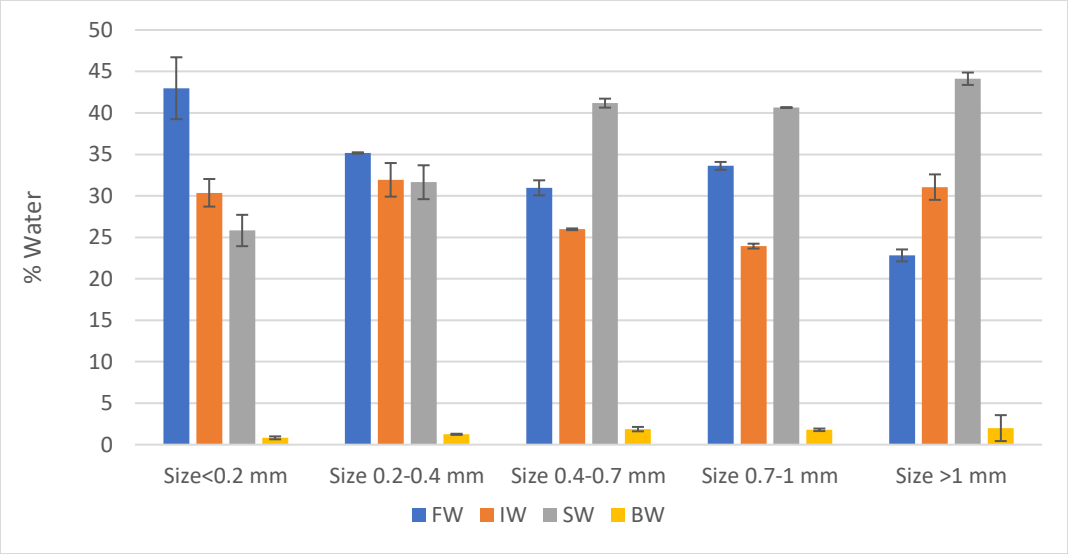

Figure 24: Impact of granule size on different water fractions

5.0 Chapter 4: Conclusion

Granular sludge is beginning to intrigue utilities and plant operators with its promises of fast settling biomass and advance nutrient removal capabilities. Yet, there are still uncertainties revolving around the dewatering potential for this type of sludge. An understanding of the water fractions existing within and around granular sludge, and how they are influenced by the structure and physical properties is needed to fully adopt this process into mainstream use.

This study aimed to increase the understanding of granular sludge dewaterability by developing a method which utilizes the principles behind drying curves and the technology of thermogravimetric analysis to observe changes in drying rates for identification of free, interstitial, surface and bound water. TGA operated at a temperature of 25°C provided ideal distinctions between each water fraction. This data was used to calculate the percentage of water

existing as free, interstitial, surface and bound within the granular sludge samples. After applying this method, granular sludge data was compared to activated sludge having been prepared in the same manner, and granular sludge had less free and interstitial water for samples that were untreated, settled for 30 minutes, and centrifuged for 30 minutes at 1000g. However, granular sludge exhibited higher percentages of surface and bound water when compared to activated sludge.

Filterability measured in terms of CST was also compared for granular sludge and activated sludge, and results showed that CST, a good indicator of the free water content and filterability of sludge, was substantially improved for granular sludge when compared to activated sludge. The CST results supported the findings established by TGA analysis for granular sludge.

Furthermore, TGA analysis was used to observe the changes in water types after polymer addition and mechanical dewatering. Granular sludge exerted less of a demand for polymer than activated sludge and had higher % cake after mechanical dewatering. Additionally, after TGA testing of the dewatered cake samples, activated sludge was shown to have increases in surface and bound water after polymer addition; while granular sludge, most likely due to less polymer added, showed an increase in only bound water.

Phase two of this study provided a fundamental understanding of some of the physico-chemical properties that affect the dewatering process of aerobic granular sludge. The findings indicate that higher TB-EPS content can improve the overall sludge-water separation, aiding in the reduction of free water. However, this is not the case with high amounts of loosely bound EPS which resulted in increased free and interstitial water. Moreover, granular sludge particle

size provided an additional factor affecting the distribution of water within granular sludge. As particle diameter increased, an increase in surface water and a decline in interstitial water was clearly revealed.

The findings from this research provide a detailed look into the distribution of water fractions existing in granular sludge, and how they are affected by major physio-chemical aspects related to granular sludge. This work begins the journey to greater understanding of granular sludge solids handling, which has been sorely lacking from the scientific community.

5.1 Future Directions:

For future studies it is essential to understand the impacts of digestion on aerobic granular sludge. Evidence in the deterioration of dewatering for activated sludge is well established in literature; however, due to the increased amount of EPS involved with the stability and integrity of aerobic granular sludge, an understanding of dewatering after digestion is needed, due to the release of the nonbiodegradable EPS and cations. Additionally, different mechanical dewatering processes and polymers will influence the dewatering performance of aerobic granular sludge. Therefore, these avenues of research must also be explored to select the units and conditioners that will aid in the best removal of water for aerobic granular sludge.

References

Adav, S. S. and D.-J. Lee (2008). "Extraction of extracellular polymeric substances from aerobic granule with compact interior structure." Journal of hazardous materials **154**(1): 1120-1126.

Adav, S. S., D.-J. Lee, K.-Y. Show and J.-H. Tay (2008). "Aerobic granular sludge: Recent advances." Biotechnology Advances **26**(5): 411-423.

Al-Halbouni, D., J. Traber, S. Lyko, T. Wintgens, T. Melin, D. Tacke, A. Janot, W. Dott and J. Hollender (2008). "Correlation of EPS content in activated sludge at different sludge retention times with membrane fouling phenomena." Water Research **42**(6): 1475-1488.

Bennamoun, L., P. Arlabosse and A. Léonard (2013). "Review on fundamental aspect of application of drying process to wastewater sludge." Renewable and Sustainable Energy Reviews **28**(Supplement C): 29-43.

Beun, J., J. Heijnen and M. Van Loosdrecht (2001). "N-Removal in a granular sludge sequencing batch airlift reactor." Biotechnology and Bioengineering **75**(1): 82-92.

Beun, J. J., J. J. Heijnen and M. C. M. van Loosdrecht (2001). "N-Removal in a granular sludge sequencing batch airlift reactor." Biotechnology and Bioengineering **75**(1): 82-92.

Chen, G., W. Lin and D. Lee (1995). "Effects of particle sedimentation on capillary suction time (CST)." JOURNAL OF THE CHINESE INSTITUTE OF CHEMICAL ENGINEERS **26**(6): 371-377.

Chen, G., W. Lin and D. Lee (1996). "Capillary suction time (CST) as a measure of sludge dewaterability." Water Science and Technology **34**(3-4): 443-448.

Chen, Y., H. Yang and G. Gu (2001). "Effect of acid and surfactant treatment on activated sludge dewatering and settling." Water Research **35**(11): 2615-2620.

Christensen, M. L., K. Keiding, P. H. Nielsen and M. K. Jørgensen (2015). "Dewatering in biological wastewater treatment: A review." Water Research **82**(Supplement C): 14-24.

Coackley, P. and B. Jones (1956). "Vacuum sludge filtration: I. Interpretation of results by the concept of specific resistance." Sewage and Industrial Wastes **28**(8): 963-976.

Colin, F. and S. Gazbar (1995). "Distribution of water in sludges in relation to their mechanical dewatering." Water Research **29**(8): 2000-2005.

de Bruin, L. M. M., M. K. de Kreuk, H. F. R. van der Roest, C. Uijterlinde and M. C. M. van Loosdrecht (2004). "Aerobic granular sludge technology: an alternative to activated sludge?" Water Science and Technology **49**(11-12): 1-7.

De Kreuk, M., J. Heijnen and M. Van Loosdrecht (2005). "Simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge." Biotechnology and bioengineering **90**(6): 761-769.

de Kreuk, M. K., N. Kishida, S. Tsuneda and M. C. M. van Loosdrecht (2010). "Behavior of polymeric substrates in an aerobic granular sludge system." Water Research **44**(20): 5929-5938.

Deng, W., X. Li, J. Yan, F. Wang, Y. Chi and K. Cen (2011). "Moisture distribution in sludges based on different testing methods." Journal of Environmental Sciences **23**(5): 875-880.

DuBois, M., K. A. Gilles, J. K. Hamilton, P. t. Rebers and F. Smith (1956). "Colorimetric method for determination of sugars and related substances." Analytical chemistry **28**(3): 350-356.

Dulekgurgen, E., S. Ovez, N. Artan and D. Orhon (2003). "Enhanced biological phosphate removal by granular sludge in a sequencing batch reactor." Biotechnology letters **25**(9): 687-693.

Faraj, R., B. Sturm, T. Amante and M. Mosquera (2017). "Implementation of an External Selector to Improve the Stability and Nutrient Removal of Aerobic Granular Sludge with Low-Strength Municipal Wastewater." Proceedings of the Water Environment Federation **2017**(3): 461-467.

Faraj, R. A. (2014). The Effect of Slowly Biodegradable Carbon on the Morphology, Integrity and Performance of Aerobic Granular Sludge, University of Kansas.

Federation, W. E. and A. P. H. Association (2005). "Standard methods for the examination of water and wastewater." American Public Health Association (APHA): Washington, DC, USA.

Felz, S., S. Al-Zuhairy, O. A. Aarstad, M. C. van Loosdrecht and Y. M. Lin (2016). "Extraction of Structural Extracellular Polymeric Substances from Aerobic Granular Sludge." Journal of visualized experiments: JoVE(115).

Forstner, G. (2016). "Impact of Orthophosphate on Achiveable Dry Cake Solids Content and Polymer Consumption in the Dewatering Process–A Summary From Us-Phosphory Recovery Tests and Two Full Sclae Installations in Europe." Proceedings of the Water Environment Federation **2016**(3): 754-771.

Higgins, M., C. Bott, P. Schauer and S. Beightol (2014). "Does Bio–P Impact Dewatering after Anaerobic Digestion? Yes, and not in a good way!" Proceedings of the Water Environment Federation **2014**(2): 1-11.

Higgins, M. J. and J. T. Novak (1997). "Characterization of exocellular protein and its role in bioflocculation." Journal of environmental engineering **123**(5): 479-485.

Higgins, M. J. and J. T. Novak (1997). "The effect of cations on the settling and dewatering of activated sludges: laboratory results." Water Environment Research **69**(2): 215-224.

Higgins, M. J., L. A. Tom and D. C. Sobeck (2004). "Case study I: Application of the divalent cation bridging theory to improve biofloc properties and industrial activated sludge system performance—Direct addition of divalent cations." Water environment research **76**(4): 344-352.

Houghton, J. J., J. Quarmby and T. Stephenson (2001). "Municipal wastewater sludge dewaterability and the presence of microbial extracellular polymer." Water Science and Technology **44**(2-3): 373-379.

Jin, B., B.-M. Wilén and P. Lant (2004). "Impacts of morphological, physical and chemical properties of sludge flocs on dewaterability of activated sludge." Chemical Engineering Journal **98**(1): 115-126.

Karr, P. R. and T. M. Keinath (1978). "Influence of Particle Size on Sludge Dewaterability." Journal (Water Pollution Control Federation) **50**(8): 1911-1930.

Katsiris, N. and A. Kouzeli-Katsiri (1987). "Bound water content of biological sludges in relation to filtration and dewatering." Water Research **21**(11): 1319-1327.

Keey, R. B. (1978). Introduction to Industrial Drying Operations, Pergamon Press.

Knocke, W. R. and T. L. Zentkovich (1986). "Effects of Mean Cell Residence Time and Particle Size Distribution on Activated Sludge Vacuum Dewatering Characteristics." Journal (Water Pollution Control Federation) **58**(12): 1118-1123.

Kopp, J. and N. Dichtl (2000). "Prediction of full-scale dewatering results by determining the water distribution of sewage sludges." Water Science and Technology **42**(9): 141-149.

Kopp, J. and N. Dichtl (2001). "Influence of the free water content on the dewaterability of sewage sludges." Water Science and Technology **44**(10): 177-183.

Kopp, J. D.-I. and N. P. D.-I. Dichtl (1998). Influence of surface charge and exopolysaccharides on the conditioning characteristics of sewage sludge. Chemical Water and Wastewater Treatment V, Springer**:** 285-296.

Li, X. Y. and S. F. Yang (2007). "Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge." Water Research **41**(5): 1022-1030.

Li, Y., J. Zou, L. Zhang and J. Sun (2014). "Aerobic granular sludge for simultaneous accumulation of mineral phosphorus and removal of nitrogen via nitrite in wastewater." Bioresource Technology **154**(Supplement C): 178-184.

Liao, B. Q., D. G. Allen, I. G. Droppo, G. G. Leppard and S. N. Liss (2001). "Surface properties of sludge and their role in bioflocculation and settleability." Water Research **35**(2): 339-350.

Lin, Y., M. de Kreuk, M. Van Loosdrecht and A. Adin (2010). "Characterization of alginate-like exopolysaccharides isolated from aerobic granular sludge in pilot-plant." Water research **44**(11): 3355-3364.

Liu, Y. and H. H. P. Fang (2003). "Influences of Extracellular Polymeric Substances (EPS) on Flocculation, Settling, and Dewatering of Activated Sludge." Critical Reviews in Environmental Science and Technology **33**(3): 237-273.

Lowry, O., N. Rosebrough, A. Farr and R. Randall (1951). "Protein estimation by Lowry's method." J Biol Chem **193**: 265.

Mahmoud, A., J. Olivier, J. Vaxelaire and A. F. A. Hoadley (2010). "Electrical field: A historical review of its application and contributions in wastewater sludge dewatering." Water Research **44**(8): 2381-2407.

Martin-Cereceda, M., F. Jorand, A. Guinea and J. Block (2001). "Characterization of extracellular polymeric substances in rotating biological contactors and activated sludge flocs." Environmental technology **22**(8): 951-959.

McSwain, B., R. Irvine, M. Hausner and P. Wilderer (2005). "Composition and distribution of extracellular polymeric substances in aerobic flocs and granular sludge." Applied and environmental microbiology **71**(2): 1051-1057.

McSwain, B., R. Irvine and P. Wilderer (2004). "The effect of intermittent feeding on aerobic granule structure." Water Science & Technology **49**(11): 19-25.

McSwain, B., R. Irvine and P. Wilderer (2004). "The influence of settling time on the formation of aerobic granules." Water Science and Technology **50**(10): 195-202.

Mikkelsen, L. H. and K. Keiding (2002). "Physico-chemical characteristics of full scale sewage sludges with implications to dewatering." Water Research **36**(10): 2451-2462.

Mikkelsen, L. H. and K. Keiding (2002). "The shear sensitivity of activated sludge: an evaluation of the possibility for a standardised floc strength test." Water Research **36**(12): 2931-2940.

Morgan, J. W., C. F. Forster and L. Evison (1990). "A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges." Water Research **24**(6): 743-750.

Morgenroth, E., T. Sherden, M. Van Loosdrecht, J. Heijnen and P. Wilderer (1997). "Aerobic granular sludge in a sequencing batch reactor." Water research **31**(12): 3191-3194.

Moy, B. P., J. H. Tay, S. K. Toh, Y. Liu and S. L. Tay (2002). "High organic loading influences the physical characteristics of aerobic sludge granules." Letters in Applied Microbiology **34**(6): 407-412.

Müller, J. A. (2001). "Prospects and problems of sludge pre-treatment processes." Water Science and Technology **44**(10): 121-128.

Murthy, S. N. and J. T. Novak (1998). "Effects of potassium ion on sludge settling, dewatering and effluent properties." Water Science and Technology **37**(4): 317-324.

Neyens, E., J. Baeyens, R. Dewil and B. De heyder (2004). "Advanced sludge treatment affects extracellular polymeric substances to improve activated sludge dewatering." Journal of Hazardous Materials **106**(2): 83-92.

Novak, J. T., G. L. Goodman, A. Pariroo and J.-C. Huang (1988). "The blinding of sludges during filtration." Journal (Water Pollution Control Federation): 206-214.

Novak, J. T., M. E. Sadler and S. N. Murthy (2003). "Mechanisms of floc destruction during anaerobic and aerobic digestion and the effect on conditioning and dewatering of biosolids." Water Research **37**(13): 3136-3144.

Poxon, T. L. and J. L. Darby (1997). "Extracellular polyanions in digested sludge: Measurement and relationship to sludge dewaterability." Water Research **31**(4): 749-758.

Pramanik, A. (1994). Characterization of water distribution in sludges, Virginia Tech.

Pronk, M., M. K. de Kreuk, B. de Bruin, P. Kamminga, R. Kleerebezem and M. C. M. van Loosdrecht (2015). "Full scale performance of the aerobic granular sludge process for sewage treatment." Water Research **84**(Supplement C): 207-217.

Robinson, J. and W. R. Knocke (1992). "Use of Dilatometric and Drying Techniques for Assessing Sludge Dewatering Characteristics." Water Environment Research **64**(1): 60-68.

Sheng, G.-P., H.-Q. Yu and X.-Y. Li (2010). "Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review." Biotechnology Advances **28**(6): 882-894.

Skinner, S. J., L. J. Studer, D. R. Dixon, P. Hillis, C. A. Rees, R. C. Wall, R. G. Cavalida, S. P. Usher, A. D. Stickland and P. J. Scales (2015). "Quantification of wastewater sludge dewatering." Water Research **82**(Supplement C): 2-13.

Smith, J. K. and P. A. Vesilind (1995). "Dilatometric measurement of bound water in wastewater sludge." Water Research **29**(12): 2621-2626.

Smollen, M. (1990). "Evaluation of Municipal Sludge Drying and Dewatering with Respect to Sludge Volume Reduction." Water Science and Technology **22**(12): 153-161.

Sturm, B., R. Faraj, T. Amante, B. Wagner, F. Kiani and D. Waybenais (2015). "Strategies for Operating Aerobic Granular Sludge Reactors for Low-Strength Municipal Wastewater." Proceedings of the Water Environment Federation **2015**(17): 5656-5665.

Tay, J., Q. Liu and Y. Liu (2002). "Characteristics of aerobic granules grown on glucose and acetate in sequential aerobic sludge blanket reactors." Environmental technology **23**(8): 931-936.

Techobanoglous, G., F. Burton and H. Stensel (2014). Wastewater Engineering: Treatment and Reuse, 5th edn. Metcalf and Eddy, McGraw-Hill series in civil and environmental engineering. McGraw-Hill, New York.

Tsang, K. R. and P. A. Vesilind (1990). "Moisture Distribution in Sludges." Water Science and Technology **22**(12): 135-142.

Unno, H., H. Muraiso and T. Akehata (1983). "Theoretical and experimental study of factors affecting capillary suction time (CST)." Water Research **17**(2): 149-156.

Urbain, V., J. C. Block and J. Manem (1993). "Bioflocculation in activated sludge: an analytic approach." Water Research **27**(5): 829-838.

Vaxelaire, J. and P. Cézac (2004). "Moisture distribution in activated sludges: a review." Water Research **38**(9): 2215-2230.

Vesilind, P. A. (1988). "Capillary Suction Time as a Fundamental Measure of Sludge Dewaterability." Journal (Water Pollution Control Federation) **60**(2): 215-220.

Vesilind, P. A. (1994). "The Role of Water in Sludge Dewatering." Water Environment Research **66**(1): 4-11.

Wang, W., Y. Luo and W. Qiao (2010). "Possible solutions for sludge dewatering in China." Frontiers of Environmental Science & Engineering in China **4**(1): 102-107.

Wingender, J., T. R. Neu and H.-C. Flemming (1999). What are bacterial extracellular polymeric substances? Microbial extracellular polymeric substances, Springer**:** 1-19.

Winkler, M.-K. H., R. Kleerebezem, M. Strous, K. Chandran and M. C. M. van Loosdrecht (2013). "Factors influencing the density of aerobic granular sludge." Applied Microbiology and Biotechnology **97**(16): 7459-7468.

Winkler, M. K. H., R. Kleerebezem, J. G. Kuenen, J. Yang and M. C. M. van Loosdrecht (2011). "Segregation of Biomass in Cyclic Anaerobic/Aerobic Granular Sludge Allows the Enrichment of Anaerobic Ammonium Oxidizing Bacteria at Low Temperatures." Environmental Science & Technology **45**(17): 7330-7337.

Winkler, M. K. H., J. Yang, R. Kleerebezem, E. Plaza, J. Trela, B. Hultman and M. C. M. van Loosdrecht (2012). "Nitrate reduction by organotrophic Anammox bacteria in a nitritation/anammox granular sludge and a moving bed biofilm reactor." Bioresource Technology **114**: 217-223.

Wu, C. C., C. Huang and D. J. Lee (1998). "Bound water content and water binding strength on sludge flocs." Water Research **32**(3): 900-904.

Yang, S.-f. and X.-y. Li (2009). "Influences of extracellular polymeric substances (EPS) on the characteristics of activated sludge under non-steady-state conditions." Process Biochemistry **44**(1): 91-96.

Yilmaz, G., R. Lemaire, J. Keller and Z. Yuan (2008). "Simultaneous nitrification, denitrification, and phosphorus removal from nutrient-rich industrial wastewater using granular sludge." Biotechnology and bioengineering **100**(3): 529-541.

Appendixes:

Appendix-A: Method for determining water fractions in granular sludge

Background:

There are many tests employed to determine the different water fractions held within wastewater sludge, however, increased curiosity regarding Thermogravimetric Analysis (TGA) application has been mounting due to ease of test, and possible prediction of full scale dewatering ability. However, drying curves, which are the fundamental principle behind TGA, have been used to quantify the moisture content in wastewater sludge and biosolids for several years. This method adopts the assumption that the evaporation of water is contingent to the bonding strength between the water molecules and solid surfaces. Changes with the drying rate of a sludge sample would provide information regarding the moisture distribution. Drying curves for activated sludge typically show four different rates of evaporation. These include a short period of increasing temperature; thus, an increased drying rate, a constant rate followed by a falling rate, and then a second decreasing rate, see figure 1 in Appendix-C.

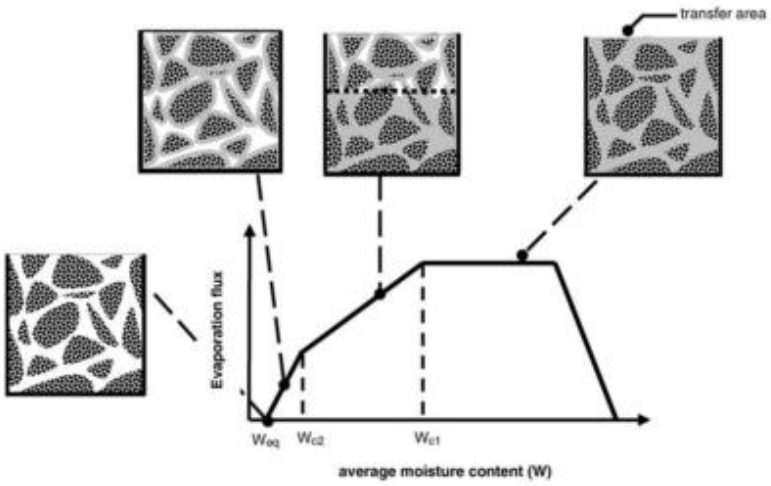

Figure A-1: Typical Drying curve taken from Vazelaire and Cezac 2004 showing the drying rates traditionally assocatied with wastewater sludge. The first increasing rate (starting from the right) indicates the increase in drying rate due to the rise in temperature to establish the isothermal temperature, the following rate is considered the evaporation of free water which shouldshow a constant rate. The two following slopes indicate intersitial and surface water respectively. The last drying rate, which should reach an evaporation flux of zero, is considered the bound water portion, which is not visible in the figure.

Materials:

Centrifuge (depending on sample preperation)

Settling cones (depending on sample preperaiton)

Filter cups

47 mm diameter glass microfiber filters (Whatman)

0.3 mm seive

SDT-Thermal analyzer (TA Instruments), see figure A-2

Q-600 Sample pans (TA Insturments)

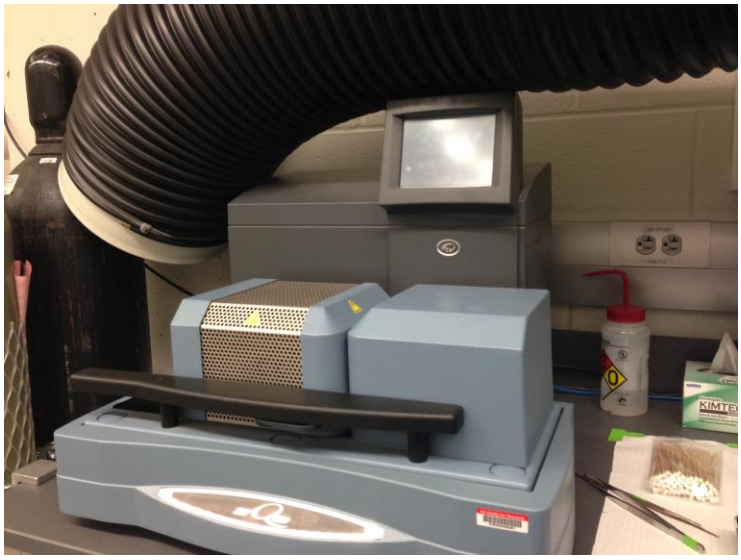

Figure A-2: Q600-Thermal analyzer

Procedure**:**

TGA Setup

1. Start up the TGA software by selecting the TGA Internet Explorer icon on the desktop, a screen will appear with the icon labeled Q600-0467 and the current temperature, select this icon.
2. Before sample preparation, allow instrument to stabilize with the empty Q-600 sample pan and the reference weight pan. There must be one reference pan located closest to the furnace on the TGA beam during all testing.
3. To stabilize the instrument, click on the control tab located on top right of software, select furnace and then select open.

4. Place sample pan on the arm located closest to you, make sure it is cleaned with ethanol or acetone and completely dried., see figure A-3
5. Close the furnace and begin establishing parameters for testing.
6. On the Summary tab located in the home page of the QSeries file, select a destination to save the output data file. Make sure the file name is the same as the "Sample Name" field.
7. On the procedure tab, under the "Test" drop down menu select "Ramp".
8. For the starting temperature, make sure the box with "Use Current" is checked.
9. In the first section for the temperature input, add a value which is close to the starting temperature of the instrument, this will typically be in the range of 18-21℃, and is shown on the screen of the furnace.
10. The final temperature should be 25℃.
11. In the "Heating Rate" section, input a heating ramp rate of 1℃/min.
12. Make sure to select the box next to "Hold time at final temperature" and in the section next to "min" add 400 to establish a hold/ testing time of 400 minutes, or longer (this time may vary based on sample).
13. In the Notes tab, make sure to have "Mass Flow Control Settings" set with #2-Air at a flow rate of 30 mL/min.
14. After establishing the parameters, begin sample preparation and allow TGA to stabilize.

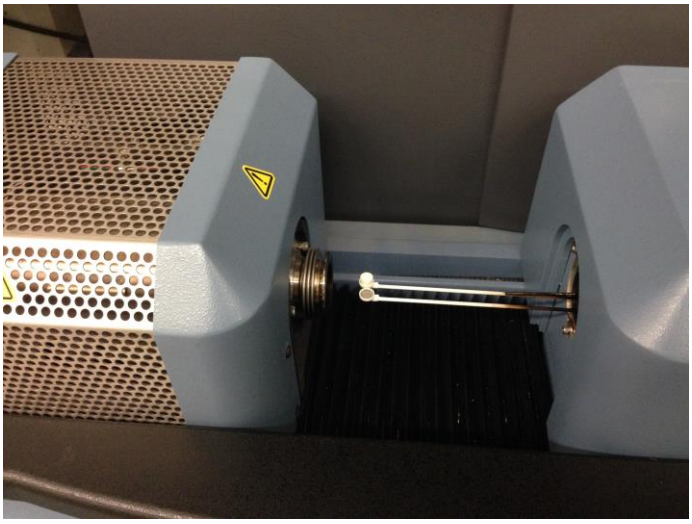

Figure A-3: picture of opened furnace with beams showing, indicating placement of sample pans

Sample Preparation

Prepare sample as needed for testing, i.e. after polymer addition, gravity thickening, or centrifuging. Sample preparation will have an impact on visualization of drying rates for each water fraction.

15. After sieving all the sampled biomass though a 0.3 mm sieve and collecting the retained particles, prepare sample as needed for testing.
16. Perform a CST test according to Standard methods with the sludge sample.
17. Before placing biomass in TGA, Tare the balances by selecting the balance icon on the QSeries home screen located at the top of the file.
18. Watch the Weight and Reference Weight values on the signal screen to see any major changes. For example, if the values begin to rise rapidly or differ more than 0.0004 from each other; if so, select Tare again and watch the change in values for at least ten seconds.
19. Once stable, open the furnace and take the sample pan out of the TGA to place your sample into. Using a 100 µL pipette, cut the tip off to the first band on the pipette tip to ensure no granules will block the path of suction, and sample 80 µL of sludge after mixing. *Note- make sure to mix well due to variable nature of the small granular sludge samples.
20. Carefully place sample in pan and make sure edges are not wet and sample is not overflowing.
21. Place pan back on beam and allow beam to stabilize, afterwards close the furnace.
22. Make sure all parameters are set and begin test by selecting the green play button on the top right-hand side of the home screen.
23. *Note- After TGA test, take dried pellet from the sample pan and perform a TSS test according to standard methods to ensure all water has evaporated from the sample. If there is a change in weight after leaving the 105℃ oven, TGA was not conducted for a long enough period. OR, ramp TGA up to 105℃ and observe any changes in weight.

Data Analysis:

24. After the test is complete, remove sample from the TGA and clean sample from the pan.
25. Select the TA Universal Analysis icon from the desktop, and on the file tab open the data file which you saved your output file.
26. This will display the DSC-TGA File with derivative of the weight on the secondary y-axis and weight (%) on the primary y-axis, see figure A-5.
27. Select file, Export Data File, then File and Plot signals, and finish the command. You will then be asked were to save the text file of your data.
28. Convert the Text file to a .csv and remove all data and inputs except the Time, Temp, Weight, Derivative of weight, Total weight (which can be found on the original txt file), and Solid weight (which is the last data point in the weight column, See figure A-4
29. Calculate the Mw/Ms for all data points by using the equation A-1 shown below, and drag calculation down for all data points.

$$\frac{Total\ Weight - Solid\ Weight}{Solid\ Weight}$$

Equation A-1: Calculation for Mw/Ms (g-water/g-solid)

30. Calculate the % Water using the equation shown below.

$$\frac{(Total\ Weight - Solid\ Weight\ )\ for\ n-time\ of\ testing}{(Total\ Weight - Solid\ Weight) - during\ start\ of\ test} \text{ X } 100$$

Equation A-2: Calculation for % Water during time of test

31. Add a column in the csv file titled "points" with consecutive numbers starting at 1 and ending at the last data point (typically around 19,000).
32. Use the data file with the r-code shown below in section B of the appendix to select sections of the curve which associate with free, interstitial, surface and bound water.

Time	Temp	weight	Deriv_wt	Total weight	solid	Mw_Ms	%_Water	points
6.11E-04	17.74177	50.83037	0.3527475	50.832	1.230954	40.29348	100	1
0.016667	17.73337	50.82657	0.3493053	50.832	1.230954	40.29039	99.99234	2
0.045833	17.72726	50.8214	0.3423328	50.832	1.230954	40.28619	99.98192	3
0.070833	17.71578	50.81706	0.337659	50.832	1.230954	40.28266	99.97317	4
0.095833	17.70932	50.81289	0.3340942	50.832	1.230954	40.27928	99.96476	5
0.125	17.70308	50.8081	0.3241147	50.832	1.230954	40.27538	99.9551	6
0.154167	17.69489	50.80335	0.3189861	50.832	1.230954	40.27153	99.94552	7
0.179167	17.68922	50.79932	0.315347	50.832	1.230954	40.26825	99.9374	8
0.204167	17.68229	50.79532	0.3123821	50.832	1.230954	40.265	99.92933	9
0.229167	17.68242	50.7914	0.3096534	50.832	1.230954	40.26182	99.92143	10
0.258333	17.67671	50.78686	0.306578	50.832	1.230954	40.25813	99.91228	11
0.2875	17.67309	50.7823	0.3035516	50.832	1.230954	40.25443	99.90308	12

Figure A-4, Sample data set *Note-use the column names exactly as shown for analysis with R

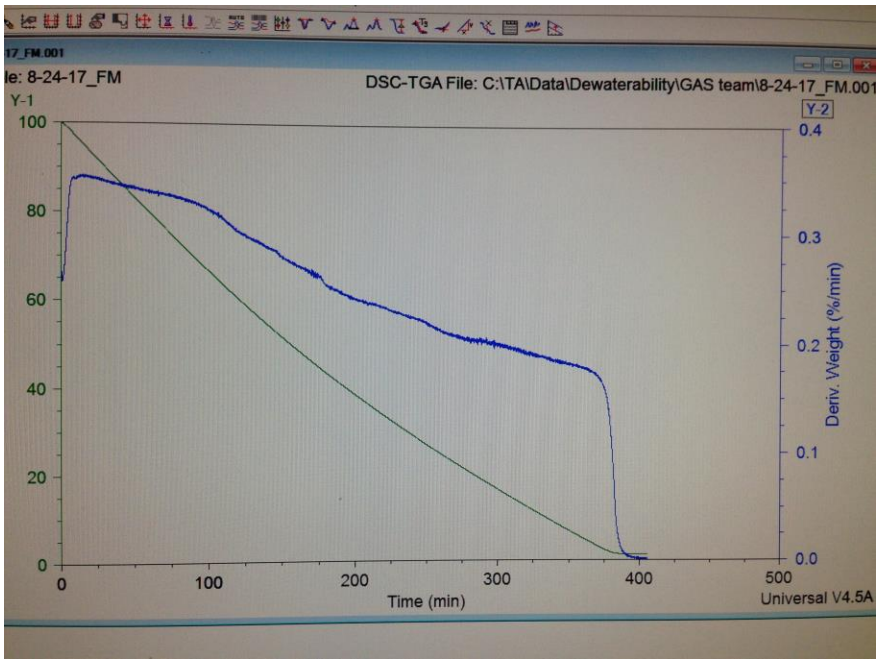

Figure A-5: Example of output file from TGA run before all plot signals are exported

Appendix B: R code for analysis of water types:

```
#Import data file “Data_file”
RData<- read.csv("Input\\”Data_file”.csv")
Deriv<- RData$Deriv_wt
Mw_Ms<- RData$Mw_Ms
RDataDF<- data.frame(Deriv,Mw_Ms)
head(RDataDF)
```

```
#plot data file and identify points of transitions for starting from right to left
plot.new()
plot(Mw_Ms,Deriv,main="July 19th", xlab="Mw/Ms (g/g)", ylab="Drying Rate (%/,min)")
DFRAW<- data.frame(Mw_Ms,Deriv)
points <- identify(DFRAW) ;points(Mw_Ms)
inflecPoints<-data.frame(points)
```

```

```{r}

#creating a data frame of the selected points and the point number associated based on the column points

head(inflecPoints)

df_points=data.frame(Mw_Ms,Deriv,"points"=RData$points)  #Creating datafram of the Mw_Ms_g_g (in non log scale) with the points values

mer_points<-merge(df_points, inflecPoints, by = c("points"))   #Matching point values with X valus (g/g)

mer_points
```

```{r}

#making a new plot with added lines at transition points

plot.new()

plot(Mw_Ms,Deriv, main="July 19th", xlab="Mw/Ms (g/g)", ylab="Drying Rate (%/,min)")


#insterting slopes and ablines to plot

abline(v=mer_points$Mw_Ms, col="purple")


```

#subseting data and creating linear regressions with data

DFslopes<-data.frame(mer_points)

DFslopes

FData<-subset(RData, Mw_Ms>DFslopes[2,2]&Mw_Ms<DFslopes[1,2])

IData<-subset(RData,Mw_Ms>DFslopes[3,2]&Mw_Ms<DFslopes[2,2])

SData<-subset(RData,Mw_Ms>DFslopes[4,2]&Mw_Ms<DFslopes[3,2])

BData<-subset(RData,Mw_Ms>DFslopes[5,2]&Mw_Ms<DFslopes[4,2])
```

```
lines(FData$Mw_Ms,FData$Deriv_wt, col="red")

lines(IData$Mw_Ms,IData$Deriv_wt, col="green")

lines(SData$Mw_Ms,SData$Deriv_wt, col="purple")

lines(BData$Mw_Ms,BData$Deriv_wt, col="orange")
```

```{r}
#adding slopes & R2

Flm<-lm(Deriv_wt~Mw_Ms, data=FData)

FR2<-summary(Flm)$r.squared

Ilm<-lm(Deriv_wt~Mw_Ms, data=IData)

IR2<-summary(Ilm)$r.squared


Slm<-lm(Deriv_wt~Mw_Ms, data=SData)

SR2<-summary(Slm)$r.squared

Blm<-lm(Deriv_wt~Mw_Ms, data=BData)

BR2<-summary(Blm)$r.squared
```

```{r}
#Creating Dataframe with all slopes and y-intercepts

Slopes<- data.frame(Flm$coefficients[[2]], Ilm$coefficients[[2]],
Slm$coefficients[[2]],Blm$coefficients[[2]])

R2<- data.frame( "Free"=summary(Flm)$r.squared, "Intersitial"=summary(Ilm)$r.squared,
"Surface"=summary(Slm)$r.squared, "Bound"=summary(Blm)$r.squared, row.names = "R
Squared")

Slopes_2<-data.frame("Free"=Slopes$Flm.coefficients..2..,
"Intersitial"=Slopes$Ilm.coefficients..2.., "Surface"=Slopes$Slm.coefficients..2..,
"Bound"=Slopes$Blm.coefficients..2.., row.names = "Slope")

DataDF<-rbind(Slopes_2,R2)

DataDF
```

```{r}

# Creating Dataframe of % Water

Percent_Water<- RData$X._Water

Water =data.frame( Percent_Water,"points"=RData$points)  #Creating datafram

Water_2<-merge(Water, inflecPoints, by = c("points"))   #Matching point values with X valus (g/g)

Water_2


Freewater<-Water_2[1,2]-Water_2[2,2]

InterWater<-Water_2[2,2]-Water_2[3,2]

SWater<-Water_2[3,2]-Water_2[4,2]

BWater<-Water_2[4,2]-Water_2[5,2]

```

```{r}

#Creating Dataframe of water types in g-water/g-solid

DFPercent<-data.frame("Free"=Freewater,"Intersitial"=InterWater,"Surface"=SWater, "Bound"=BWater, row.names = "% Water")

DFPercent

Fg<-mer_points[1,2]-mer_points[2,2]

Ig<-mer_points[2,2]-mer_points[3,2]

Sg<-mer_points[3,2]-mer_points[4,2]

Bg<-mer_points[4,2]-mer_points[5,2]

#WHg<-mer_24th[5,2]-mer_24th[6,2]

Watergrams<-data.frame("Free"=Fg,"Intersitial"=Ig,"Surface"= Sg, "Bound"=Bg, row.names = "Mw_Ms (g/g)")

Watergrams

```

```{r}

#combining all values into one table and exporting it as a csv
```

```
Summary<-rbind(Slopes_2,R2,DFPercent,Watergrams)

Summary

write.csv(Summary,
'C:/Users/There/OneDrive/Documents/R/Inflection/TGA_FM/Output_2/"title_of_table".csv')
```

Appendix C: Statistical analysis for determining whether slopes are significantly different. Method used- Students T' test

Suspect slopes were tested using a statistical model created in excel to ensure slopes were statistically different. In Figure B-1, equations are shown next to variables. The null hypothesis suggests that the slopes have no statistical difference. However, a p-value of less than 0.05 suggests that the user can reject the null hypothesis.

Mw_Ms	Deriv_wt
44.04315	0.354975
44.0396	0.354316
44.03538	0.353626
44.0312	0.353229
44.02774	0.353113
44.02429	0.353169
44.02011	0.35346
44.0159	0.353891
44.01235	0.354106
44.0088	0.354488

Mw_Ms	Deriv_wt
33.57119	0.332382
33.56723	0.332104
33.5633	0.331732
33.56001	0.331405
33.55675	0.331269
33.55283	0.331019
33.54889	0.331038
33.54562	0.331224
33.54232	0.331589
33.5384	0.331846

Slope1	0.002255	"=SLOPE(y,x)"
Slope2	0.004837	"=SLOPE(y,x)"
SE1	0.001428	"=STEYX(y,x)"
SE2	0.007679	"=STEYX(y,x)"
SD1	3.027611	"=STDEV(X)"
SD2	6.801451	"=STDEV(X)"
n1	2774	"=COUNT(x)"
n2	8658	"=COUNT(x)"

Sb_1	8.95848E-06	"=(S_y,x)/(S_x*sqrt(n-1))"
Sb_2	1.21339E-05	"=(S_y,x)/(S_x*sqrt(n-1))"
Sb1-Sb2	1.50827E-05	"=SQRT(sb_1^2+sb_2^2)"
t	-171.2283366	"=(b1-b2)/(sb1-sb2)"
df	11428	"=(n1+n2-4)"
alpha	0.05	
p-value	0.00	"=TDIST(|t|,df,2)"
t-crit	1.96017159	"=TINV(alpha,df)"
slopes different?	yes	"=yes if p-value < alpha"

Figure B-1: Example set for t-test with interstitial and surface water, with equations shown next to variables